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## 16. ANIMAL BIOTECHNOLOGY

**Unit 1: Cell Biology**

Origin and evolution of cells, Prokaryotic and eukaryotic cell architecture, Structure of cell, Molecular organization and functions of cell membrane, organization and functions of the cytoplasm, cell organelles, endoplasmic reticulum, ribosomes, Golgi complex, mitochondria, lysosomes, nucleus, nucleolus and sub-nuclear structures, Protein secretion and targeting. Cell division, cell cycle, Cell growth and differentiation. Apoptosis and molecular pathways, different techniques used to study cells. Cell Membrane transport, transport of small molecules, macromolecules and particles, exocytosis and endocytosis, nuclear transport, protein synthesis and sorting, Cell signaling and Signal Transduction, steroid receptor super family – Neurotransmitters, peptide hormones and regulation of cellular functions.

**Unit 2: Molecular Biology**

History and scope of molecular biology – Discovery of DNA and evidence for DNA as the genetic material - structure of DNA, RNA and proteins – Organization of prokaryotic and eukaryotic genome, DNA replication - genetic code - transcription, RNA processing and alternative splicing – Translation in prokaryotes and eukaryotes - Regulation of gene expression. DNA replication in prokaryotic and eukaryotic cells. Structure and functions of DNA polymerases. Molecular mechanisms of DNA repair. Synthesis and processing of different types of RNA. RNA polymerases. Protein biosynthesis. Genetics of mitochondria and plasmids. Transposons and intervening sequences. Minisatellites and microsatellites. Molecular mechanism of spontaneous and induced mutations. Recombination in bacteria, viruses and fungi. Molecular mechanism of genetic recombination, transduction, transformation and conjugation.

**Unit 3: Gene Structure and Regulation of Gene Expression**

Repeated and non-repeated DNA sequences. Structure and function of gene. Expression of genetic information, transcription - mechanism of transcription in prokaryotes and eukaryotes, transcription unit, regulatory sequences and enhancers, transcription factors, post- transcriptional modifications, DNA-protein and protein-protein interactions. Genetic code, Overlapping genes, Mechanism of translation and its control, post-translational modifications, Control of gene expression in prokaryotes and eukaryotes, Gene mining.

**Unit 4: Genetic Engineering and Recombinant DNA Technology**

Enzymes used in molecular biology and recombinant DNA research, Isolation and purification of DNA and RNA from prokaryotes and eukaryotes. Reverse transcription and cDNA synthesis, Restriction endonucleases, Generation of DNA fragments, Cloning and expression vectors-plasmids, cosmids, phages, phagemid, shuttle vectors, BAC, YAC, MAC. Eukaryotic vectors-viral vectors (vaccinia, retro, adeno, baculo) and yeast expression system, Cloning and expression in prokaryotic and eukaryotic hosts. DNA libraries (genomic and cDNA), Screening and characterization of DNA clones. Transformation of bacterial cells, Transfection in animal cells, *In situ* mutagenesis. Site directed mutagenesis, Antibody engineering, Production of diagnostics, therapeutics and vaccines using r-DNA technology, Nano-delivery system, Genetically modified organisms, Genetic manipulation of microbes, single cell protein, Reverse genetics, RNA interference (RNAi, siRNA, peptide nucleic acid), Gene silencing, Biosafety and biosecurity aspects and regulations associated with recombinant DNA technology research, Ethical issues related to use of biotechnology products, Patenting and Intellectual Property Rights.

**Unit 5: Cell culture and Hybridoma Technology**

History of cell culture development, Methods of sterilization, Different tissue culture techniques including primary culture, Continuous cell lines- anchorage dependent and independent cell lines, Organ culture, Cell bank. Factors that affecting the growth of cells. Development and applications of cell and organ culture techniques. Nutrient requirements for cells of animal and fish origin. Media for culturing cells, Growth supplements. Stationary, roller and suspension culture techniques. Large-scale production of cells using bioreactors, micro-carriers and perfusion techniques. Characterization and maintenance of cells/cell lines, karyotyping, cryopreservation and revival. Detection of contaminants in cell cultures. Cell viability and cytotoxicity assays. Isolation and culture of lymphocytes. Micro-manipulation of cells, Cell fusion, somatic cell hybrids, sub-cloning of cells. Principles and methods of hybridoma technology, Production and characterization of monoclonal antibodies, epitope mapping of antibodies, application of monoclonal antibodies in diagnostic, cancer research and therapeutics, Antibody engineering: designer antibodies, Chimeric and humanized monoclonal antibodies, Recombinant antibodies.

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**Unit 6: Reproductive Biotechnology and Related Techniques**

Assisted Reproductive Technology, Multiple Ovulation Embryo Transfer (MOET), *in-vitro* fertilization, Micro assisted fertilization, Embryo culture, Micromanipulation of gametes and embryos, preservation of embryos and oocytes, Embryo splitting, sexing, transfer and their applications, Superovulation, Semen sexing technology. Cryopreservation of gamete and embryos. *In vitro* fertilization, Embryo cloning, nuclear transplantation, Transgenic technology, Production of transgenic animals and gene pharming, Gene knockout techniques. Identification and transfer of genes influencing production and disease resistance. Stem cells and applications in animal health and production. Somatic cell nuclear transfer and application. Isolation and characterization of embryonic stem cells. Different applications of embryonic stem cells.

**Unit 7: Molecular Biology Techniques & Molecular Diagnostics**

Quantitation of protein and nucleic acids. Gel electrophoretic techniques. Isolation of plasmids. Molecular cloning, Nucleic acid probes, GFP from jelly fish and its applications. Nucleic acid hybridization including in *situ* hybridization and FISH techniques. Autoradiography. Blotting techniques. Nucleic acid sequencing methods including next generation sequencing (NGS), Protein purification methods. RFLP, RAPD and AFLP. DNA fingerprinting. Single Nucleotide Polymorphism (SNP). Polymerase Chain Reaction (PCR) and its variants, Real-time PCR, molecular beacons. DNA/Protein Microarray techniques, Biosensors, Nano diagnostics, Mass spectrometry, Bead based assays and lateral-flow technology. Solid phase peptide synthesis and applications of synthetic peptides.

**Unit 8: Bioinformatics in biotechnology**

Introduction, Database searching - Biological Data Acquisition, Retrieval methods for DNA sequence, protein sequence and protein structure information, General Introduction of Biological Database, Protein and nucleic acid data banks, sequence alignment, sequence editing, phylogenetic tree, Genome analysis using *in silico* tools, Introduction to concept chemo informatics computer aided drug Design–basic principles, Docking, QSAR, Immuno-informatic, Artificial intelligence and applications in drug designing, animal health and production, Genome databases.

**Unit 9: Animal Genomics and Genome Editing Technology**

Genome organization in eukaryotes-Chromosome numbers in farm animals, Importance of repetitive DNA, Minisatellite and microsatellite based fingerprinting techniques. Metagenomics, RNASeq analysis, Gene mapping in livestock, DNA markers, Role of MHC in disease resistance, Genes influencing production traits, Mitochondrial DNA of farm animals, Genome editing techniques–Zinc fingers, TALENS, Mega nucleases and CRISPR – Cas and importance in livestock improvement.

**Unit 10: Vaccine Technology**

Current trends in vaccine development against animal pathogens (virus, bacteria, parasites); Molecular approaches for vaccine development including: recombinant vaccines, vectored vaccines, Marker vaccines, DNA vaccines, genetically manipulated live vaccines etc.; Plant expression system-based vaccines, idiotype and synthetic peptide-based vaccines. Vaccinomics, Adversomics, Systems Vaccinology, reverse vaccinology, Structural Vaccinology, DIVA Vaccines for animal disease, Novel immunomodulators and vaccine delivery systems: Immunomodulators including cytokines and new adjuvants; delivery of immunogens through liposomes, microspheres, ISCOMS, nanotechnology based vaccine delivery, Clinical trials of vaccine and its regulation, Vaccine formulation: pharmacopeia requirements; Vaccine qualities and its control; Large scale vaccine production technology, Use of modern adjuvants in vaccines.



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# CHAPTER 01
# Cell Biology

2004

**1.** Which theory hypothesizes that self replicating ribonucleic acid molecules were precursors to all current life on Earth?
(A) RNA world hypothesis
(B) Endosymbiotic theory
(C) Chemical evolution theory
(D) Panspermia hypothesis

2009

**2.** In the context of chemical evolution, what was the primary energy source proposed for synthesizing organic compounds in the primitive Earth atmosphere?
(A) Geothermal heat from deep-sea vents
(B) Spontaneous oxidation of inorganic iron
(C) Ultraviolet radiation and lightning
(D) Decay of radioactive isotopes

2018

**3.** The endosymbiotic theory strongly relies on which unique characteristic of mitochondria to support its evolutionary claim?
(A) Possession of circular DNA and 70S ribosomes
(B) Presence of a single, continuous membrane
(C) Dependence on nuclear DNA for all protein synthesis
(D) Inability to undergo binary fission independently

2012

**4.** Identify the INCORRECT statement regarding the RNA world hypothesis.
(A) RNA can function both as a genetic material and a biological catalyst.
(B) Ribozymes provide evidence that RNA can catalyze chemical reactions.
(C) The synthesis of peptide bonds by rRNA in modern ribosomes supports this hypothesis.
(D) RNA is chemically more stable than DNA, making it an ideal long-term storage molecule.

2011

**5.** Which of the following best describes the initial atmosphere required for the Oparin-Haldane model of chemical evolution?
(A) Highly oxidizing, consisting primarily of molecular oxygen and atmospheric carbon dioxide
(B) Reducing, composed mainly of methane, ammonia, hydrogen, and water vapor
(C) Neutral, consisting largely of nitrogen gas, noble gases, and traces of water
(D) Oxidizing, dominated by sulfur dioxide, nitrous oxide, and high concentrations of ozone

2014

**6.** What is the most significant limitation of the Miller-Urey experiment in proving the origin of life?
(A) It failed to produce any biologically relevant amino acids necessary for protein synthesis.
(B) It utilized ultraviolet light sources instead of the intended high-voltage electrical discharge.
(C) It only demonstrated the synthesis of complex nucleic acids, but failed to synthesize proteins.
(D) It assumed a highly reducing atmosphere, which early Earth may not have had.

2012

**7.** According to the endosymbiotic theory, chloroplasts evolved from which specific group of organisms?
(A) Free-living aerobic proteobacteria
(B) Symbiotic methanogenic archaea
(C) Chemolithotrophic sulfur bacteria
(D) Free-living photosynthetic cyanobacteria

2022

**8.** Following are two statements, one labeled as Assertion (A) and the other as Reason (R). Select the correct answer using the codes given below.
**Assertion (A):** Mitochondria and chloroplasts are considered semi-autonomous organelles.
**Reason (R):** They contain their own genetic material and protein synthesis machinery but rely on the nuclear genome for some essential proteins.
(A) Both A and R are true and R is the correct

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explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2014
**9.** In the context of chemical evolution, coacervates are best defined as which of the following?
(A) Crystalline structures of amino acids formed under extreme heat and pressure conditions
(B) Droplets of organic polymers that form spontaneously in a liquid phase
(C) The first fossilized remnants of primitive prokaryotic cells found in ancient rock strata
(D) Inorganic clay templates used for early RNA polymerization and molecular stabilization

2010
**10.** Which experimental evidence most directly contradicts the theory of spontaneous generation and supports biogenesis?
(A) The Miller-Urey electric discharge experiment
(B) Pasteur's swan-neck flask experiment
(C) Fox's synthesis of proteinoid microspheres
(D) Woese's discovery of the Archaea domain

2007
**11.** What is the primary functional advantage of the proposed transition from an RNA-based world to a DNA-based world?
(A) Deoxyribose and the absence of a 2'-OH group make DNA less prone to hydrolysis.
(B) DNA possesses versatile catalytic properties that RNA molecules completely and inherently lack.
(C) The double-stranded structure of DNA allows for specialized, high-efficiency selF- cleavage.
(D) DNA replication does not require any enzymatic proteins, unlike RNA replication.

2025
**12.** Which process is considered the likely driving force behind the secondary endosymbiosis of plastids in certain eukaryotic lineages?
(A) Simultaneous fusion of two identical prokaryotic cells during an environmental stress event
(B) Viral transfer of photosynthetic genes into an animal cell via specialized transduction pathways
(C) Engulfment of a photosynthetic eukaryote by a heterotrophic eukaryote
(D) Spontaneous compartmentalization of cytoplasmic enzymes into primitive membrane-bound sacs

2008
**13.** Which cellular structure is strictly characteristic of prokaryotic cell architecture and entirely absent in eukaryotes?
(A) Phospholipid bilayer membrane
(B) Ribosomal protein synthesis machinery
(C) Genomic DNA organized into linear chromosomes
(D) Peptidoglycan-based cell wall

2006
**14.** What constitutes the fundamental advantage of cellular compartmentalization in eukaryotic cells?
(A) It eliminates the biological necessity for a semi-permeable phospholipid plasma membrane.
(B) It ensures all genetic material is equally distributed during simple binary fission processes.
(C) It allows for the spatial separation and optimization of competing biochemical pathways.
(D) It prevents the entry of viral vectors into the complex internal cytoplasmic matrix.

2005
**15.** Identify the correct pair distinguishing plant cells from animal cells.
(A) Plant cells have centrosomes; animal cells lack them.
(B) Plant cells contain a large central vacuole; animal cells typically have small or no vacuoles.
(C) Plant cells lack a plasma membrane; animal cells possess one.
(D) Plant cells possess lysosomes as their primary digestive organelle; animal cells use glyoxysomes.

2002
**16.** Which structural feature provides motility in some prokaryotes and differs completely in protein composition from its eukaryotic counterpart?
(A) Cilia composed of tubulin dimers
(B) Pseudopodia driven by actin microfilaments
(C) Pili consisting of integral membrane glycoproteins
(D) Flagella composed of the protein flagellin

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2012

**17.** Which statement correctly compares the genomic organization of prokaryotes and eukaryotes?
(A) Prokaryotic genomes typically consist of a single circular chromosome located in the nucleoid.
(B) Eukaryotic genomes are mostly circular, whereas prokaryotic genomes are strictly linear and short.
(C) Prokaryotic DNA is heavily complexed with histones, unlike eukaryotic DNA strands.
(D) Eukaryotes lack extrachromosomal DNA completely, whereas prokaryotes possess specialized circular plasmids.

2015

**18.** Plasmids in prokaryotic cells primarily serve which biological function?
(A) They are the primary site of oxidative phosphorylation in the cell.
(B) They encode all essential housekeeping genes required for basic survival and reproduction.
(C) They carry accessory genes that may provide selective advantages, such as antibiotic resistance.
(D) They regulate the synthesis of the peptidoglycan cell wall during the process of binary fission.

2018

**19.** What is the structural basis for the difference between Gram-positive and Gram-negative prokaryotic cell envelopes?
(A) Gram-negative cells feature a thin peptidoglycan layer situated between an inner and outer membrane.
(B) Gram-negative cells have a thick peptidoglycan layer and no outer membrane.
(C) Gram-positive cells have a thin peptidoglycan layer enclosed by an outer lipopolysaccharide membrane.
(D) Gram-positive cells lack peptidoglycan entirely, relying on a rigid protein S-layer.

2003

**20.** Which eukaryotic organelle is most analogous to the prokaryotic plasma membrane in terms of hosting electron transport chain complexes?
(A) The rough endoplasmic reticulum
(B) The membrane of the central vacuole
(C) The cis-Golgi network
(D) The inner mitochondrial membrane

2014

**21.** Following are some statements regarding prokaryotic and eukaryotic cells:
I. Prokaryotes divide by binary fission.
II. Eukaryotic ribosomes in the cytosol are of the 70S type.
III. Eukaryotes possess membrane-bound organelles.
Which of the above statement(s) is/are INCORRECT?
(A) I and II
(B) II only
(C) I and III
(D) III only

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2003

**22.** The nucleolus is a characteristic feature of eukaryotic cells. What is its primary functional role?
(A) Storage of extrachromosomal plasmid DNA sequences
(B) Segregation of sister chromatids during the active mitotic phase
(C) Synthesis and assembly of ribosomal RNA and ribosomal subunits
(D) Enzymatic degradation of misfolded nuclear proteins via the proteasome

2009

**23.** Which cytoskeletal element is highly developed in animal cells to maintain cell shape but is functionally replaced by the cell wall in plant cells?
(A) Microtubules
(B) Actin microfilaments
(C) Kinetochore fibers
(D) Intermediate filaments

2015

**24.** What is the main structural component of the fungal cell wall, distinguishing it from plant and prokaryotic cell walls?
(A) Chitin
(B) Cellulose
(C) Peptidoglycan
(D) Lignin

2008

**25.** The fluid mosaic model of the cell membrane was originally proposed by which scientists?
(A) Watson and Crick
(B) Singer and Nicolson
(C) Schleiden and Schwann
(D) Davson and Danielli

2024

**26.** Match the membrane component in List I with its primary characteristic in List II:
List I: (Membrane Component)

P. Phospholipid
Q. Cholesterol
R. Integral protein
S. Peripheral protein

List II: (Primary Characteristic)
1. Spans the hydrophobic core
2. Modulates membrane fluidity
3. Bound to the membrane surface
4. Forms the fundamental bilayer matrix

(A) P-4, Q-2, R-1, S-3
(B) P-1, Q-4, R-3, S-2
(C) P-4, Q-1, R-2, S-3
(D) P-2, Q-3, R-4, S-1

2023

**27.** According to the fluid mosaic model, membrane fluidity is primarily determined by which biochemical property?
(A) The proportion of unsaturated fatty acid chains in phospholipids
(B) The degree of glycosylation on peripheral membrane proteins
(C) The total concentration of integral transmembrane channels
(D) The rapid flip-flop movement of glycolipids across the bilayer

2006

**28.** What characterizes the molecular organization of lipid rafts within the cell membrane?
(A) High concentrations of unsaturated lipids and assorted peripheral membrane proteins
(B) Depletion of cholesterol and enrichment of specialized, freely diffusing enzymes
(C) Microdomains rich in cholesterol, sphingolipids, and specific receptor proteins
(D) Exclusive assemblies of integrins disconnected from the fundamental lipid bilayer

2019

**29.** How are integral membrane proteins structurally adapted to their position in the lipid bilayer?
(A) They consist entirely of hydrophilic amino acids to interact with the aqueous cytosol.
(B) They are covalently linked to the phosphate heads without entering the lipid core.
(C) They lack tertiary structure, existing only as disordered peptide chains.
(D) They possess hydrophobic domains that interact with the fatty acid tails of the bilayer.

2006

**30.** Which molecular movement within the lipid bilayer is thermodynamically unfavorable and requires enzymatic assistance by flippases?
(A) Lateral diffusion within the same phospholipid leaflet
(B) Rotational movement around the lipid's primary long axis
(C) Transverse diffusion (flip-flop) from one leaflet to the other
(D) Flexion of the hydrocarbon tails

2025

**31.** Following are some statements regarding membrane lipid asymmetry:
I. Glycolipids are predominantly found on the extracellular leaflet.
II. Phosphatidylserine is normally concentrated in the cytosolic leaflet.
III. Lipid asymmetry is static and cannot be altered during cellular events like apoptosis.
IV. Scramblases maintain asymmetry by strictly unidirectional lipid transport.

Which of the above statement(s) is/are correct?
(A) I and III
(B) I and II
(C) II and IV
(D) I, II and IV

2019

**32.** Which class of membrane proteins can be readily removed from the membrane by mild treatments, such as high salt concentration or pH change, without disrupting the lipid bilayer?
(A) Transmembrane alpha-helical proteins
(B) Lipid-anchored proteins
(C) Multipass beta-barrel proteins
(D) Peripheral membrane proteins

2013

**33.** Which of the following best describes the function of glycocalyx on the outer surface of animal cell membranes?
(A) It facilitates cell-cell recognition, adhesion, and protection.
(B) It forms a rigid structural barrier similar to a plant cell wall.
(C) It acts as the primary channel for active transport of ions.
(D) It synthesizes ATP using a proton gradient.

2008

**34.** What effect does increasing the cholesterol content have on a cell membrane at physiological body temperatures?
(A) It drastically increases membrane

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permeability to water.
(B) It causes the immediate separation of the lipid bilayer into two independent monolayers.
(C) It restricts the movement of phospholipid fatty acid chains, decreasing fluidity.
(D) It promotes the rapid degradation of integral membrane proteins.

2007
**35.** Identify the INCORRECT statement regarding membrane carbohydrates.
(A) They are situated exclusively on the cytosolic face of the plasma membrane.
(B) They are commonly covalently bound to proteins to form glycoproteins.
(C) They play a crucial role in immune system recognition.
(D) They contribute to the overall negative charge of the cell surface.

2002
**36.** Which structural motif is most frequently utilized by multipass integral membrane proteins to span the hydrophobic core of the bilayer?
(A) Hydrophilic beta-sheets
(B) Disulfide bridges
(C) Unstructured loops
(D) Hydrophobic alpha-helices

2015
**37.** The eukaryotic cytoskeleton is primarily composed of which three main types of protein filaments?
(A) Motor proteins such as myosin, kinesin, and dynein
(B) Actin filaments, microtubules, and intermediate filaments
(C) Extracellular proteins like fibrillin, elastin, and collagen
(D) Cell surface receptors like tubulin, flagellin, and integrin

2004
**38.** Which fundamental cellular process is predominantly driven by the assembly and disassembly of microfilaments?
(A) Separation of sister chromatids during the mitotic phase of the cell cycle
(B) Structural formation of the nuclear lamina beneath the envelope
(C) Amoeboid movement and formation of pseudopodia
(D) Anchoring of large organelles in fixed positions within the cytosol

2006
**39.** Microtubules are hollow cylindrical polymers assembled from which basic protein subunit?
(A) Globular actin (G-actin) monomers
(B) Fibrous keratin protofibrils
(C) Vimentin tetrameric complexes
(D) Alpha and beta tubulin heterodimers

2006
**40.** What is the functional significance of the dynamic instability exhibited by microtubules?
(A) It allows for the rapid remodeling of the cytoskeleton, essential for cell division.
(B) It ensures permanent structural rigidity of the cell membrane.
(C) It prevents the binding of motor proteins to the microtubule lattice during transport.
(D) It restricts microtubules strictly to the perinuclear region of the cytoplasm.

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2012
**41.** Following are some statements regarding intermediate filaments:
I. They are involved in maintaining cellular mechanical strength.
II. They exhibit structural polarity like actin filaments.
III. Keratin and lamin are examples of intermediate filaments.
IV. They are directly responsible for the movement of cilia.
V. They primarily utilize ATP for their assembly.
Which of the above statements are INCORRECT?
(A) II, IV and V
(B) I, II and III
(C) I, III and V
(D) II, III, IV and V

2013
**42.** During muscle contraction, microfilaments interact specifically with which motor protein to generate force?
(A) Kinesin
(B) Myosin
(C) Dynein
(D) Nexin

2002
**43.** Which of the following intermediate filaments is responsible for supporting the inner structural integrity of the nuclear envelope?
(A) Nuclear lamins
(B) Neurofilaments
(C) Desmin
(D) Glial fibrillary acidic protein (GFAP)

2025

**44.** Motor proteins transport cellular cargo along microtubules. Which statement accurately describes their directionality?
(A) Both kinesin and dynein move exclusively towards the plus end.
(B) Dyneins generally move towards the plus end, while kinesins move towards the minus end.
(C) Kinesins generally move towards the plus end, while dyneins move towards the minus end.
(D) Motor proteins move bidirectionally without preference for filament polarity.

2025

**45.** In animal cells, the microtubule-organizing center (MTOC) is typically associated with which structure?
(A) The nucleolus
(B) The basal body of microvilli
(C) The kinetochore
(D) The centrosome

2001

**46.** What chemical compound acts as the energy source for the polymerization of actin microfilaments?
(A) GTP
(B) CTP
(C) ATP
(D) UTP

2009

**47.** Which drug specifically targets microtubules by binding to tubulin subunits and preventing their polymerization, thereby arresting mitosis?
(A) Phalloidin
(B) Cytochalasin
(C) Latrunculin
(D) Colchicine

2014

**48.** Identify the correct pair connecting a cytoskeletal element to its structural diameter, listed in ascending order of size.
(A) Microfilaments — 7 nm
(B) Intermediate filaments — 10 nm
(C) Microtubules — 25 nm
(D) Centrioles — 250 nm

2023

**49.** The rough endoplasmic reticulum (RER) is distinguished from the smooth endoplasmic reticulum (SER) primarily by the presence of which bound structures?
(A) Ribosomes
(B) Lysosomes
(C) Peroxisomes
(D) Dictyosomes

2022

**50.** Which cellular process is uniquely localized to the smooth endoplasmic reticulum in eukaryotic cells?
(A) Synthesis of integral membrane proteins
(B) Addition of N-linked oligosaccharides to nascent peptides
(C) Biosynthesis of membrane lipids and steroid hormones
(D) Generation of ATP via oxidative phosphorylation

2008

**51.** Select the correct sequence of the secretory pathway for a newly synthesized digestive enzyme.
(A) Smooth ER → Golgi → Lysosome → Plasma membrane
(B) Rough ER → Golgi → Secretory vesicle → Plasma membrane
(C) Nucleus → Rough ER → Smooth ER → Plasma membrane
(D) Cytosol → Golgi → Rough ER → Secretory vesicle

2025

**52.** What role does the sarcoplasmic reticulum, a specialized form of smooth ER in muscle cells, play in muscle contraction?
(A) It functions as a dynamic intracellular storage site for calcium ions.
(B) It synthesizes the major actin and myosin structural filaments.
(C) It degrades metabolic lactic acid built up during muscle exertion.
(D) It provides ATP directly to the myosin heads for contraction.

2012

**53.** The initial step of N-linked protein glycosylation in the rough ER involves the transfer of an oligosaccharide precursor from which lipid anchor?
(A) Dolichol phosphate
(B) Phosphatidylinositol
(C) Sphingomyelin
(D) Cardiolipin

2024

**54.** Identify the INCORRECT statement regarding the endoplasmic reticulum.

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(A) The ER membrane is continuous with the outer nuclear membrane.
(B) Detoxification of drugs in the liver is heavily reliant on the smooth ER.
(C) The lumen of the ER constitutes a chemically distinct environment from the cytosol.
(D) All translation of mRNA in a eukaryotic cell occurs on the rough ER.

2008

**55.** What happens to newly synthesized proteins in the rough ER that fold incorrectly?
(A) They are translocated back to the cytosol for degradation by the proteasome (ERAD pathway).
(B) They are immediately secreted out of the cell to avoid potentially toxic intracellular aggregation.
(C) They are packaged into lysosomes directly from the ER using specialized bypass transport vesicles.
(D) They are permanently stored within the ER lumen as inert inclusion bodies to protect the cytoplasm.

2003

**56.** Which resident ER protein acts as a critical chaperone to assist in the proper folding of nascent polypeptide chains?
(A) Clathrin protein complexes
(B) Ubiquitin signaling chains
(C) Kinesin motor molecules
(D) BiP (Binding Immunoglobulin Protein)

2016

**57.** In cells heavily engaged in lipid metabolism, such as the Leydig cells of the testis, which organelle is expected to be highly developed?
(A) Rough ER
(B) Nucleolus
(C) Contractile vacuole
(D) Smooth ER

2008

**58.** By what mechanism do secretory proteins translocate into the lumen of the rough ER?
(A) Co-translational translocation via the Sec61 channel
(B) Post-translational import through nuclear pore complexes
(C) Direct simple diffusion across the phospholipid bilayer
(D) Phagocytosis by the ER membrane

2015

**59.** The smooth ER in liver hepatocytes is rich in enzymes belonging to which family, crucial for drug detoxification?
(A) Acid hydrolases
(B) Cytochrome P450
(C) Caspases
(D) ATP synthases

2005

**60.** How does the ER primarily contribute to the formation of other membrane-bound organelles like the Golgi apparatus?
(A) By replicating itself through binary fission
(B) By budding off transitional transport vesicles
(C) By exporting soluble proteins through the cytosol
(D) By direct physical elongation into Golgi cisternae

2004

**61.** Following is a list of functional ribosomal sites involved in mRNA translation:
1. Peptidyl (P) site
2. Exit (E) site
3. Aminoacyl (A) site

Which of the following represents the correct sequence of tRNA movement through a ribosome?
(A) 3 → 1 → 2
(B) 1 → 2 → 3
(C) 2 → 1 → 3
(D) 3 → 2 → 1

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2010

**62.** Which of the following correctly describes the subunit composition of a eukaryotic 80S ribosome?
(A) 50S and 30S subunits
(B) 40S and 40S subunits
(C) 60S and 20S subunits
(D) 60S and 40S subunits

2015

**63.** Ribosomal RNA (rRNA) represents what approximate percentage of total cellular RNA in a typical eukaryotic cell?
(A) 80%
(B) 5%
(C) 20%
(D) 50%

2023

**64.** What is the catalytic function of the 28S rRNA molecule within the large eukaryotic ribosomal subunit?
(A) It acts as a helicase to unwind mRNA.

(B) It recognizes the start codon on the mRNA transcript to initiate synthesis.
(C) It attaches activated amino acids to their respective tRNA molecules.
(D) It catalyzes the formation of peptide bonds (peptidyl transferase activity).

2004

**65.** The assembly of ribosomal subunits in eukaryotes takes place primarily in which subcellular compartment?
(A) The rough ER lumen
(B) The nucleolus
(C) The cytoplasm
(D) The Golgi apparatus

2009

**66.** Identify the correct statement regarding prokaryotic ribosomes.
(A) They contain exactly the same rRNA species as eukaryotic ribosomes.
(B) They are exclusively bound to the prokaryotic plasma membrane.
(C) They do not utilize tRNA during translation.
(D) They are composed of a 50S large subunit and a 30S small subunit.

2016

**67.** Which specific rRNA molecule is found in the small subunit (40S) of the eukaryotic ribosome?
(A) 18S rRNA
(B) 5S rRNA
(C) 16S rRNA
(D) 28S rRNA

2010

**68.** During the initiation of translation in eukaryotes, the small ribosomal subunit first binds to which structure on the mRNA?
(A) The Shine-Dalgarno sequence
(B) The 3' poly-A tail
(C) The 5' cap (m7G)
(D) The TATA box

2017

**69.** Which class of antibiotics inhibits bacterial protein synthesis by specifically binding to the 30S ribosomal subunit and causing misreading of the mRNA?
(A) Penicillins
(B) Aminoglycosides (e.g., Streptomycin)
(C) Macrolides like Erythromycin or Azithromycin
(D) Cephalosporins targeting the bacterial cell wall

2024

**70.** What is a polyribosome (or polysome)?
(A) A cluster of ribosomes assembled in the nucleolus waiting for nuclear export processes
(B) A giant ribosome complex capable of translating genomic DNA directly into proteins
(C) A specialized membrane channel where inactive ribosomes are targeted for degradation
(D) A complex of multiple ribosomes simultaneously translating a single mRNA molecule

2024

**71.** The 5S rRNA in eukaryotes is unique because, unlike other rRNAs, it is synthesized by which enzyme?
(A) RNA polymerase III
(B) RNA polymerase I
(C) RNA polymerase II
(D) Peptidyl transferase

2011

**72.** In terms of spatial organization during translation, where is the newly growing polypeptide chain typically directed if it possesses an ER signal sequence?
(A) Into the nucleoplasm via the nuclear pore complex
(B) Through a translocon channel into the ER lumen
(C) Directly to a lysosome for enzyme-mediated cleavage
(D) Into the mitochondrial matrix

2024

**73.** The Golgi apparatus in plant cells is often organized into separate, unconnected stacks known as:
(A) Peroxisomes
(B) Plasmodesmata
(C) Tonoplasts
(D) Dictyosomes

2001

**74.** Which specific face of the Golgi apparatus is generally oriented toward the endoplasmic reticulum and serves as the entry face for transport vesicles?
(A) The trans face
(B) The medial network
(C) The cis face
(D) The secretory face

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2024

**75.** A primary function of the Golgi apparatus is the post-translational modification of proteins. Which modification is uniquely characteristic of the Golgi?
(A) Initial attachment of N-linked oligosaccharides
(B) Synthesis of the polypeptide chain
(C) Complex O-linked glycosylation
(D) Ubiquitination for degradation

2008

**76.** Following are two statements, one labeled as Assertion (A) and the other as Reason (R). Select the correct answer using the codes given below.
**Assertion (A):** The Golgi apparatus plays a critical role in directing lysosomal enzymes to their correct destination.
**Reason (R):** It phosphorylates mannose residues on lysosomal enzymes to form mannose-6-phosphate, which acts as a targeting signal.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2009

**77.** Which vesicular transport mechanism is responsible for retrograde transport from the Golgi apparatus back to the endoplasmic reticulum?
(A) COPII-coated vesicles
(B) Clathrin-coated vesicles
(C) COPI-coated vesicles
(D) Caveolae

2008

**78.** In the structural organization of the Golgi, what occurs in the trans-Golgi network (TGN)?
(A) Proteins undergo initial folding and disulfide bond formation within the lumen.
(B) Vesicles from the ER initially dock and fuse with the incoming membrane.
(C) Proteins are sorted and packaged into specific vesicles destined for different cellular locations.
(D) Ribosomes bind to the membrane surface to translate late-stage mRNA transcripts.

2025

**79.** Identify the INCORRECT statement concerning the Golgi apparatus.
(A) It synthesizes the core oligosaccharide tree linked to asparagine.
(B) It synthesizes major extracellular matrix polysaccharides in plant cells.
(C) It is composed of flattened membrane-enclosed sacs called cisternae.
(D) It exhibits clear morphological and functional polarity.

2023

**80.** The process by which transport vesicles move sequentially from the cis-cisterna to the trans-cisterna while the cisternae themselves remain stationary is described by which model?
(A) Cisternal maturation model
(B) Signal hypothesis
(C) Vesicular transport model
(D) Fluid mosaic model

2018

**81.** Which enzyme group in the Golgi apparatus is responsible for transferring specific sugar residues to growing oligosaccharide chains?
(A) Glycosidases
(B) Glycosyltransferases
(C) Proteases
(D) Phosphatases

2001

**82.** Which organelle is primarily responsible for synthesizing sphingomyelin and glycolipids in animal cells?
(A) Nucleus
(B) Rough ER
(C) Peroxisome
(D) Golgi apparatus

2012

**83.** The continuous flow of membrane vesicles from the ER to the Golgi to the plasma membrane is referred to as the:
(A) Secretory (exocytic) pathway
(B) Endocytic pathway
(C) Autophagic pathway
(D) Retrograde transport pathway

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2002

**84.** Proteins that are targeted to the plasma membrane from the Golgi apparatus typically travel in which type of vesicle?
(A) Retrograde COPI-coated vesicles
(B) Anterograde COPII-coated vesicles
(C) Primary or secondary lysosomes
(D) Secretory vesicles or constitutive transport vesicles

2013
**85.** The invaginations of the inner mitochondrial membrane that significantly increase its surface area are known as:
(A) Cristae
(B) Cisternae
(C) Thylakoids
(D) Grana

2007
**86.** Match the mitochondrial compartment in List I with its corresponding process/feature in List II:
List I: (Mitochondrial Compartment)
P. Outer membrane
Q. Inner membrane
R. Intermembrane space
S. Matrix
List II: (Process/Feature)
1. Accumulation of protons for chemiosmosis
2. Citric acid cycle (Krebs cycle)
3. Presence of porin channels
4. Electron transport chain complexes
(A) P-3, Q-4, R-1, S-2
(B) P-1, Q-2, R-3, S-4
(C) P-3, Q-1, R-4, S-2
(D) P-4, Q-3, R-2, S-1

2012
**87.** Which structural feature accounts for the high permeability of the outer mitochondrial membrane compared to the inner membrane?
(A) Presence of large pore-forming proteins called porins
(B) High concentration of phospholipid cardiolipin in the membrane
(C) Lack of a functional phospholipid bilayer in the outer shell
(D) Presence of specific ATP synthase ion-conducting channels

2016
**88.** The enzymes responsible for the oxidation of pyruvate and fatty acids are predominantly located in which mitochondrial region?
(A) Inner membrane
(B) Outer membrane
(C) Intermembrane space
(D) Matrix

2025
**89.** Which lipid is highly enriched in the inner mitochondrial membrane and is essential for the optimal function of numerous respiratory enzymes?
(A) Cardiolipin
(B) Cholesterol
(C) Phosphatidylserine
(D) Sphingomyelin

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2011
**90.** During oxidative phosphorylation, the electron transport chain pumps protons from the matrix into which compartment?
(A) The cytosol
(B) The inner mitochondrial membrane
(C) The mitochondrial matrix
(D) The intermembrane space

2012
**91.** Identify the INCORRECT statement regarding mitochondrial DNA (mtDNA).
(A) It is typically circular and lacks histones.
(B) It encodes only a fraction of the proteins needed for mitochondrial function.
(C) Multiple copies of mtDNA are present within a single mitochondrion.
(D) It follows strictly Mendelian inheritance in most mammals.

2002
**92.** The $F_1$ portion of the ATP synthase complex, which contains the catalytic sites for ATP synthesis, projects into which mitochondrial region?
(A) Intermembrane space
(B) Cytosol
(C) Matrix
(D) Outer membrane

2009
**93.** What is the ultimate electron acceptor at the end of the mitochondrial electron transport chain?
(A) Cytochrome c molecules
(B) Nicotinamide adenine dinucleotide
(C) Molecular oxygen ($O_2$)
(D) Liquid water molecules ($H_2O$)

2023
**94.** Mitochondria play a key non-respiratory role in initiating which cellular process by releasing cytochrome c into the cytosol?
(A) Glycolysis
(B) Mitosis
(C) Exocytosis
(D) Apoptosis

2016
**95.** The synthesis of new mitochondria inside a cell occurs exclusively through which process?
(A) Growth and binary fission of pre-existing mitochondria
(B) De novo assembly from basic cytosolic

protein components
(C) Budding from the specialized smooth endoplasmic reticulum
(D) Engulfment of exogenous bacteria via active phagocytosis

2016
**96.** Which protein complex in the inner mitochondrial membrane DOES NOT directly pump protons across the membrane?
(A) Complex I (NADH dehydrogenase)
(B) Complex III (Cytochrome bc1 complex)
(C) Complex IV (Cytochrome c oxidase)
(D) Complex II (Succinate dehydrogenase)

2012
**97.** Which structurally defining feature of chloroplasts is entirely absent in mitochondria?
(A) An internal system of interconnected membranous sacs called thylakoids
(B) An inner membrane
(C) An outer membrane
(D) A fluid-filled matrix or stroma containing genomic DNA and ribosomes

2016
**98.** Plastids that specialize in synthesizing and storing starch in non-photosynthetic plant tissues are called:
(A) Chromoplasts
(B) Elaioplasts
(C) Proteinoplasts
(D) Amyloplasts

2023
**99.** In plant cells, the large central vacuole is surrounded by a specialized single membrane called the:
(A) Tonoplast
(B) Plasmalemma
(C) Symplast
(D) Apoplast

2014
**100.** What is a primary function of peroxisomes in eukaryotic cells?
(A) Synthesis of ATP through the process of glycolysis
(B) Intracellular digestion of worn-out cellular organelles
(C) Synthesis of ribosomal RNA in the sub-nuclear region
(D) Degradation of long-chain fatty acids via beta-oxidation

2016
**101.** Following are some statements regarding microbodies:
I. Peroxisomes contain catalase to degrade hydrogen peroxide.
II. Glyoxysomes are specialized peroxisomes found only in animals.
III. Glyoxysomes convert stored lipids into carbohydrates during plant seed germination.
Which of the above statement(s) is/are correct?
(A) I and III
(B) I and II
(C) II and III
(D) I, II and III

2016
**102.** Where do the light-dependent reactions of photosynthesis occur within the chloroplast?
(A) The stroma
(B) The intermembrane space
(C) The thylakoid membrane
(D) The outer membrane

2001
**103.** The enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is primarily localized in which chloroplast compartment?
(A) Stroma
(B) Thylakoid lumen
(C) Outer membrane
(D) Grana

2008
**104.** Which unique capability is provided to plant seedlings by the glyoxylate cycle occurring in glyoxysomes?
(A) The ability to fix atmospheric nitrogen for protein synthesis
(B) The ability to perform photosynthetic reactions in complete darkness
(C) The ability to directly absorb complex sucrose molecules from the soil
(D) The ability to synthesize glucose from acetyl-CoA derived from fat stores

2012
**105.** Plant vacuoles play a crucial role in maintaining cell turgor pressure. This is primarily driven by the active transport of which substance into the vacuole?
(A) Cellulose
(B) Solutes and ions
(C) Starch grains
(D) Large structural proteins

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2025

**106.** Identify the INCORRECT statement regarding plastid interconversion.
(A) Proplastids can differentiate into any type of mature, functional plastid.
(B) Chloroplasts can convert into chromoplasts during the fruit ripening stage.
(C) Amyloplasts can transform into chloroplasts when exposed to external light.
(D) Plastid interconversions strictly require genetic material transfer from the nucleus.

2025

**107.** Which specialized organelle is heavily involved in photorespiration alongside chloroplasts and mitochondria?
(A) The peroxisome
(B) The Golgi apparatus
(C) The lysosome
(D) The smooth ER

2010

**108.** The fluid contained within the chloroplast, surrounding the thylakoid network, is termed the:
(A) Matrix
(B) Cytosol
(C) Stroma
(D) Lumen

2016

**109.** Lysosomes function optimally in which type of biochemical environment?
(A) Acidic (pH ~4.5 to 5.0)
(B) Highly basic (pH ~9.0)
(C) Neutral (pH ~7.0)
(D) Highly variable depending on the cytosolic pH

2017

**110.** How do lysosomes maintain their internal pH against the concentration gradient?
(A) Simple diffusion of protons out of the lysosome into the cytosol
(B) Action of an ATP-dependent vacuolar-type $H^+$ pump (V-ATPase)
(C) Transport of basic amino acids into the cytosolic compartment
(D) Hydrolysis of intra-lysosomal water molecules

2012

**111.** Following are some statements regarding lysosomes:
I. Primary lysosomes contain active enzymes and actively degrading material.
II. Secondary lysosomes form by the fusion of a primary lysosome with an endosome or phagosome.
III. Lysosomal enzymes are synthesized in the rough ER.
IV. Lysosomes rely heavily on oxidases for digestion.
Which of the above statement(s) is/are INCORRECT?
(A) I and IV
(B) II and III
(C) I and II
(D) III and IV

2025

**112.** The enzymes contained within lysosomes are collectively categorized as:
(A) Acid hydrolases
(B) Alkaline phosphatases
(C) Mixed-function oxidases
(D) ATP synthases

2014

**113.** What is the process by which a cell targets its own damaged organelles or cytoplasmic components for lysosomal degradation?
(A) Phagocytosis
(B) Pinocytosis
(C) Autophagy
(D) Exocytosis

2018

**114.** Which structure forms initially during macroautophagy to enclose the cytoplasmic material targeted for destruction?
(A) Autophagosome
(B) Endosome
(C) Phagolysosome
(D) Residual body

2025

**115.** What is the fate of indigestible materials remaining within a lysosome after the digestion process is complete?
(A) They are immediately reutilized by the cell's nucleus for transcription.
(B) They are converted into mitochondrial ATP.
(C) They are transferred back to the Golgi for recycling into new enzymes.
(D) They remain in vesicles called residual bodies, which may undergo exocytosis or accumulate as lipofuscin.

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2008

**116.** A deficiency in specific lysosomal enzymes results in a buildup of undigested macromolecules, leading to which class of conditions?
(A) Autoimmune diseases
(B) Mitochondrial myopathies
(C) Peroxisomal biogenesis disorders involving enzyme mislocalization
(D) Lysosomal storage disorders (e.g., Tay-Sachs disease)

2002

**117.** Identify the correct mechanism that prevents lysosomal enzymes from digesting the lysosome's own membrane.
(A) The internal membrane proteins are highly glycosylated, providing a protective carbohydrate shield.
(B) The membrane is made of a unique synthetic polymer resistant to acid.
(C) The enzymes are strictly bound to the membrane and cannot access it.
(D) The lysosomal membrane is composed solely of rigid cholesterol molecules that cannot be hydrolyzed.

2021

**118.** Which statement best defines a "primary lysosome"?
(A) A lysosome actively digesting a phagocytosed bacterium within the cytoplasm
(B) A mature autophagolysosome containing degraded organelles
(C) A lysosome that has accumulated lipofuscin pigment over time
(D) A newly budded vesicle from the Golgi containing inactive or pre-active digestive enzymes

2025

**119.** What specific signal directs acid hydrolases to the lysosome rather than being secreted outside the cell?
(A) A mannose-6-phosphate (M6P) tag added in the Golgi
(B) A C-terminal KDEL sequence
(C) An N-terminal nuclear localization signal peptide
(D) A ubiquitin chain added in the cytosolic compartment

2012

**120.** During starvation, which cellular process is robustly upregulated to provide essential amino acids and nutrients by breaking down non-essential cellular components?
(A) Apoptosis
(B) Receptor-mediated endocytosis
(C) Transcription
(D) Autophagy

2007

**121.** Following are some statements regarding the nucleus and sub-nuclear organization:
I. The nuclear envelope consists of a double membrane.
II. The outer nuclear membrane is continuous with the rough ER.
III. The nuclear lamina is located on the cytoplasmic face of the nuclear envelope.
IV. The nucleolus is bounded by a single continuous membrane.
V. Nuclear pore complexes regulate transport in and out of the nucleus.
Which of the above statement(s) is/are correct?
(A) I, II and V
(B) I, III and IV
(C) II, III and V
(D) I, IV and V

2002

**122.** The structural framework that provides mechanical support to the inner nuclear membrane is known as the:
(A) Nuclear matrix
(B) Nucleolus
(C) Chromatin network
(D) Nuclear lamina

2017

**123.** The transport of large macromolecules, such as mRNA and assembled ribosomal subunits, across the nuclear envelope occurs strictly through which structures?
(A) Voltage-gated ion channels
(B) Flippase transporters
(C) Simple diffusion across the bilayer
(D) Nuclear pore complexes (NPCs)

2009

**124.** What is the primary function of Cajal bodies, discrete sub-nuclear structures found in the nucleoplasm?
(A) Assembly and modification of small nuclear ribonucleoproteins (snRNPs)
(B) DNA replication
(C) Storage of lipid droplets
(D) Breakdown of misfolded nuclear proteins via specialized proteasomal pathways

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2022
**125.** Identify the INCORRECT statement regarding the nucleolus.
(A) It is the site of transcription for most ribosomal RNA genes.
(B) It disassembles during the prophase of mitosis.
(C) It is enclosed by a selectively permeable phospholipid bilayer.
(D) It contains large loops of DNA known as nucleolar organizer regions (NORs).

2021
**126.** Which class of proteins primarily organizes the highly condensed structure of chromatin within the nucleus?
(A) Cohesins
(B) Transcription factors
(C) Lamins
(D) Histones

2007
**127.** Euchromatin differs from heterochromatin in that euchromatin is:
(A) Less condensed and actively undergoing transcription
(B) Highly condensed and transcriptionally inactive in the cell
(C) Found exclusively at the centromeres and telomeres of DNA
(D) Devoid of any associated histone protein structures

2011
**128.** What is the function of the nuclear matrix (or nucleoskeleton)?
(A) It generates ATP for nuclear transport.
(B) It translates mRNA into nuclear proteins.
(C) It serves as a structural scaffold organizing chromatin and aiding in transcription and replication.
(D) It synthesizes the lipid components of the double-layered nuclear envelope.

2004
**129.** Small molecules ($< 40$ kDa) typically move through the nuclear pore complex via which mechanism?
(A) Active transport requiring ATP
(B) Carrier-mediated endocytosis
(C) Active transport requiring Ran-GTP
(D) Passive diffusion

2018
**130.** Nuclear lamina mutations are directly linked to which group of genetic diseases?
(A) Lysosomal storage diseases
(B) Glycogen storage diseases
(C) Peroxisomal biogenesis disorders and lipid defects
(D) Progeria and various muscular dystrophies

NODIA

2024
**131.** During the cell cycle, the nuclear envelope typically breaks down and re-forms. In which phase does the nuclear envelope re-assemble around the newly segregated chromosomes?
(A) Prophase
(B) Metaphase
(C) Telophase
(D) Anaphase

2025
**132.** The nucleolus consists of three distinct regions: the fibrillar center, the dense fibrillar component, and the granular component. What happens primarily in the granular component?
(A) rRNA genes are stored.
(B) Initial transcription of the 45S pre-rRNA occurs in this region.
(C) Transfer RNA is synthesized.
(D) Pre-ribosomal particles undergo assembly and maturation.

2024
**133.** Which of the following transport mechanisms requires the direct expenditure of cellular energy (ATP) to move molecules against their concentration gradient?
(A) Primary active transport
(B) Simple diffusion
(C) Facilitated diffusion
(D) Osmosis

2021
**134.** Simple diffusion across the cell membrane is most restricted for which type of molecule?
(A) Small nonpolar molecules (e.g., $O_2$, $CO_2$)
(B) Small uncharged polar molecules (e.g., Hzzz2O, ethanol)
(C) Small charged ions (e.g., $Na^+$, $Cl^-$)
(D) Large hydrophobic molecules (e.g., steroid hormones)

2007
**135.** What defines facilitated diffusion compared to simple diffusion?
(A) It utilizes specific integral membrane proteins (channels or carriers) to cross the membrane.
(B) It requires ATP hydrolysis.
(C) It moves substances against their natural

concentration gradient.
(D) It only occurs in plant cells.

2024

**136.** Following are two statements, one labeled as Assertion (A) and the other as Reason (R). Select the correct answer using the codes given below.

**Assertion (A):** The $Na^+/K^+$ ATPase pump helps maintain the resting membrane potential of animal cells.

**Reason (R):** It transports 3 $Na^+$ ions out of the cell and 2 $K^+$ ions into the cell for every ATP hydrolyzed, creating an electrochemical gradient.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2008

**137.** Which statement best describes the function of aquaporins?

(A) They are specialized channel proteins that rapidly facilitate the diffusion of water across the membrane.
(B) They actively pump water molecules out of the cell against its existing osmotic gradient.
(C) They transport protons alongside water molecules to maintain the internal cellular pH levels.
(D) They regulate the active transport of glucose in the kidney.

2024

**138.** ABC transporters represent a large family of membrane proteins that perform what specific function?

(A) They transport molecules primarily using the energy of an existing ion gradient.
(B) They passively channel amino acids into the mitochondria.
(C) They exclusively mediate the import of water-soluble vitamins.
(D) They utilize ATP-binding cassettes to actively transport various substrates across membranes.

2010

**139.** The movement of glucose from the gut lumen into intestinal epithelial cells against its concentration gradient, driven by the downhill movement of $Na^+$, is an example of:

(A) Secondary active transport (symport)
(B) Primary active transport
(C) Facilitated diffusion
(D) Secondary active transport (antiport)

2012

**140.** Osmosis is formally defined as the movement of water across a semi-permeable membrane from an area of:

(A) Higher solute concentration to an area of lower solute concentration
(B) Lower water potential to higher water potential
(C) Higher osmotic pressure to lower osmotic pressure
(D) Lower solute concentration to higher solute concentration

2019

**141.** Identify the INCORRECT statement concerning ion channels.

(A) They can be gated by changes in membrane voltage.
(B) They can be gated by the binding of a specific ligand to the receptor.
(C) They exhibit high specificity for the individual ions they transport.
(D) They transport ions at rates much slower than carrier proteins.

2013

**142.** If a human red blood cell is placed in a hypertonic saline solution, what is the expected physiological outcome?

(A) The cell will lose water and shrink (crenation).
(B) The cell will swell and eventually burst (lyse).
(C) The cell will maintain its normal biconcave shape.
(D) The cell will actively pump salt out to maintain equilibrium.

2018

**143.** Which of the following is an example of an antiport transport system?

(A) The co-transport of $Na^+$ and glucose together into the intestinal epithelial cell
(B) The passive movement of $K^+$ out of the cell through non-gated leak channels
(C) The transport of water through aquaporins
(D) The $Na^+/Ca^{2+}$ exchanger, which moves $Ca^{2+}$ out of the cell while allowing $Na^+$ to enter

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2010
**144.** The multidrug resistance (MDR) protein, which can pump chemotherapeutic drugs out of cancer cells, belongs to which class of transporters?
(A) V-type ATPases
(B) Ligand-gated ion channels
(C) ABC transporters
(D) Solute carrier (SLC) group

2005
**145.** The cellular process by which materials are exported out of the cell via the fusion of secretory vesicles with the plasma membrane is termed:
(A) Endocytosis
(B) Transcytosis
(C) Exocytosis
(D) Phagocytosis

2006
**146.** Match the bulk transport mechanism in List I with its appropriate description in List II:
List I: (Bulk Transport Mechanism)
P. Phagocytosis
Q. Pinocytosis
R. Receptor-mediated endocytosis
S. Exocytosis
List II: (Description)
1. Release of neurotransmitters into the synapse
2. Non-specific uptake of extracellular fluids
3. Engulfment of large particles or whole cells
4. Highly specific uptake of ligands using clathrin
(A) P-3, Q-2, R-4, S-1
(B) P-2, Q-3, R-1, S-4
(C) P-3, Q-4, R-2, S-1
(D) P-1, Q-2, R-3, S-4

2007
**147.** Which specific protein coats the vesicles formed during receptor-mediated endocytosis?
(A) Clathrin
(B) COP I
(C) COP II
(D) Caveolin

2010
**148.** In receptor-mediated endocytosis, the pinch-off of the invaginated clathrin-coated pit from the plasma membrane requires the action of which GTPase enzyme?
(A) Ran
(B) Rab
(C) Dynamin
(D) Ras

2014
**149.** Macrophages and neutrophils primarily utilize which bulk transport mechanism to eliminate invading pathogenic bacteria?
(A) Phagocytosis
(B) Pinocytosis
(C) Exocytosis
(D) Constitutive secretion

2009
**150.** What differentiates constitutive exocytosis from regulated exocytosis?
(A) Constitutive exocytosis transports large proteins, whereas regulated exocytosis transports only small ions and waste.
(B) Constitutive exocytosis only occurs in neurons.
(C) Regulated exocytosis utilizes COPII vesicles, whereas constitutive exocytosis uses clathrin-mediated pathways.
(D) Constitutive exocytosis operates continuously without an external signal, whereas regulated exocytosis requires a specific trigger.

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2017
**151.** Select the correct sequence of events during receptor-mediated endocytosis of LDL.
(A) Ligand binding → Clathrin pit formation → Dynamin cleavage → Uncoating → Fusion with endosome
(B) Clathrin pit formation → Uncoating → Ligand binding → Dynamin cleavage → Fusion with endosome
(C) Fusion with endosome → Ligand binding → Uncoating → Clathrin pit formation → Dynamin cleavage
(D) Ligand binding → Dynamin cleavage → Clathrin pit formation → Fusion with endosome → Uncoating

2015
**152.** In bulk transport, what is the role of SNARE proteins?
(A) They form the structural coat of the vesicle.
(B) They bind to ATP to drive vesicle movement along microtubules in the cytosol.
(C) They pinch off the vesicle from the donor membrane.
(D) They mediate the specific docking and fusion of vesicles with their target membranes.

2018
**153.** Pinocytosis is often referred to as "cell drinking." Which statement correctly describes this process?
(A) It involves the continuous, non-specific

uptake of small droplets of extracellular fluid.
(B) It is highly selective for specific macromolecules entering the cell.
(C) It relies heavily on large actin-driven pseudopodia for engulfment.
(D) It is utilized solely for expelling toxic metabolic waste products.

2011
**154.** Familial hypercholesterolemia is typically caused by a defect in which bulk transport process?
(A) Phagocytosis of red blood cells
(B) Receptor-mediated endocytosis of low-density lipoproteins (LDL)
(C) Exocytosis of cholesterol from the liver
(D) Transcytosis of cholesterol across the polarized blood-brain barrier

2019
**155.** Transcytosis is a specialized cellular mechanism that involves:
(A) Endocytosis on one side of a polarized cell followed by exocytosis on the opposite side
(B) The direct transport of cytosolic proteins into the nucleus via pores
(C) The fusion of multiple lysosomes to form a megavacuole structure
(D) The spontaneous bursting of secretory vesicles into the matrix

2022
**156.** The release of insulin from pancreatic beta cells in response to elevated blood glucose is a classic example of:
(A) Constitutive exocytosis
(B) Pinocytosis
(C) Phagocytosis
(D) Regulated exocytosis

2007
**157.** The signal hypothesis states that proteins destined for the secretory pathway possess an N-terminal signal sequence. Which ribonucleoprotein complex recognizes and binds this sequence in the cytosol?
(A) Signal Recognition Particle (SRP)
(B) Spliceosome
(C) Ribosome Recycling Factor complexes
(D) Sec61 complex

2024
**158.** Upon binding the signal peptide, what is the immediate effect of the Signal Recognition Particle (SRP) on translation?
(A) It drastically accelerates translation.
(B) It terminates translation and releases the truncated polypeptide into the cytosol.
(C) It targets the mRNA for immediate degradation by nucleases.
(D) It temporarily pauses translation until the ribosome docks at the ER membrane.

2025
**159.** Proteins directed to the nucleus must contain a specific amino acid sequence known as the Nuclear Localization Signal (NLS). An NLS is typically rich in which type of amino acids?
(A) Positively charged basic amino acids (Lys, Arg)
(B) Negatively charged amino acids like Asp or Glu
(C) Aromatic amino acids such as Phe, Trp, or Tyr
(D) Branched-chain amino acids (Leu, Ile, Val)

2022
**160.** Which transport protein binds to the Nuclear Localization Signal (NLS) to mediate the import of proteins into the nucleus?
(A) Exportin
(B) Clathrin
(C) Kinesin
(D) Importin

2018
**161.** Following is a list of events involved in protein targeting to the mitochondrial matrix:
1. Translocation through TIM complex
2. Translocation through TOM complex
3. Binding of presequence to import receptor
4. Cleavage of the signal sequence by matrix processing peptidase

Which of the following represents the correct sequence of events?
(A) $3 \rightarrow 2 \rightarrow 1 \rightarrow 4$
(B) $2 \rightarrow 3 \rightarrow 1 \rightarrow 4$
(C) $3 \rightarrow 1 \rightarrow 2 \rightarrow 4$
(D) $2 \rightarrow 1 \rightarrow 3 \rightarrow 4$

2025
**162.** Vesicles that mediate transport from the endoplasmic reticulum to the cis-Golgi network are coated with:
(A) Clathrin
(B) COPI
(C) Caveolin
(D) COPII

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2005
**163.** The KDEL sequence (Lys-Asp-Glu-Leu) at the C-terminus of a protein serves as a retention/retrieval signal for which cellular compartment?
(A) Endoplasmic reticulum lumen
(B) Mitochondrial matrix
(C) Lysosome
(D) Nucleus

2013
**164.** Proteins destined for the lysosome are specifically tagged in the Golgi apparatus with which molecule?
(A) Ubiquitin
(B) Mannose-6-phosphate
(C) Farnesyl lipid anchor
(D) Sialic acid

2003
**165.** The transport of proteins from the cytosol into peroxisomes requires a specific targeting signal. What is the most common peroxisomal targeting signal ($PTS_1$)?
(A) An N-terminal amphipathic alpha-helix
(B) A central patch of basic amino acids
(C) An N-terminal signal sequence cleaved by signal peptidase
(D) A C-terminal Ser-Lys-Leu (SKL) tripeptide

2011
**166.** Identify the INCORRECT statement regarding protein sorting to mitochondria.
(A) Mitochondrial proteins are synthesized completely in the cytosol before import.
(B) Proteins are transported into the mitochondria in an unfolded state.
(C) Chaperone proteins like Hsp70 are required to prevent premature folding.
(D) All mitochondrial proteins are coded by the mitochondrial genome and do not require import.

2009
**167.** Which molecular switch regulates the directionality of nuclear transport by existing in different states in the nucleus versus the cytosol?
(A) Ran GTPase
(B) Rab GTPase
(C) Rho GTPase
(D) Ras GTPase

2011
**168.** In vesicular targeting, what determines the specific specificity between a transport vesicle and its correct target membrane?
(A) The size of the transport vesicle
(B) The thickness of the clathrin coat
(C) The ATP concentration in the local cytoplasmic environment
(D) The specific pairing of v-SNAREs on the vesicle with t-SNAREs on the target membrane

2016
**169.** The standard eukaryotic cell cycle is divided into four main phases. During which phase does DNA replication strictly occur?
(A) S phase
(B) $G_1$ phase
(C) $G_2$ phase
(D) M phase

2017
**170.** Cells that have permanently or temporarily stopped dividing and exited the active cell cycle are said to be in which state?
(A) $G_1$ phase
(B) $G_2$ phase
(C) S phase
(D) $G_0$ phase

2022
**171.** Progression through the cell cycle is fundamentally driven by the cyclic activation and inactivation of which enzymes?
(A) Adenylate cyclases
(B) MAP kinases
(C) Caspases
(D) Cyclin-dependent kinases (CDKs)

2002
**172.** The $G_1$/S checkpoint (often called the Restriction Point in mammals) determines whether a cell will:
(A) Enter mitosis
(B) Commit to DNA synthesis and complete the cell cycle
(C) Undergo cytokinesis
(D) Segregate its sister chromatids into daughter cells

2025
**173.** Which tumor suppressor protein plays a critical role at the $G_1$/S checkpoint by halting the cell cycle in response to DNA damage?
(A) p53
(B) Ras
(C) BCl-2
(D) Cyclin B

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2007

**174.** The Maturation Promoting Factor (MPF), which drives a cell from $G_2$ into M phase, consists of which two core components?
(A) Cyclin D and $CDK_4$
(B) Cyclin E and CDK2
(C) p21 and p53
(D) Cyclin B and CDK1

2003

**175.** Identify the INCORRECT statement regarding cyclins.
(A) Their intracellular concentration fluctuates periodically during the cell cycle.
(B) They bind to CDKs to form active complexes.
(C) They are targeted for rapid degradation via the ubiquitin-proteasome pathway.
(D) They possess intrinsic enzymatic kinase activity.

2022

**176.** Following are two statements, one labeled as Assertion (A) and the other as Reason (R). Select the correct answer using the codes given below.
**Assertion (A):** The Spindle Assembly Checkpoint (SAC) delays the onset of anaphase until all chromosomes are properly attached to the mitotic spindle.
**Reason (R):** Unattached kinetochores generate a biochemical signal that inhibits the Anaphase-Promoting Complex/Cyclosome (APC/C).
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2016

**177.** The Anaphase-Promoting Complex (APC/C) triggers chromatid separation by ubiquitinating which protein, leading to its degradation?
(A) Securin
(B) Cohesin
(C) Separase
(D) Tubulin

2023

**178.** What is the primary functional consequence of the phosphorylation of the retinoblastoma (Rb) protein by $G_1$-CDK complexes?
(A) It induces immediate apoptosis.
(B) It releases the $E_2F$ transcription factor, enabling the transcription of S-phase genes.
(C) It strongly inhibits DNA polymerase activity.
(D) It causes the condensation of chromatin into visible chromosomal structures.

2007

**179.** DNA damage during the $G_2$ phase activates the $G_2$/M checkpoint. This pathway ultimately inhibits the activation of MPF by preserving the inhibitory phosphorylation on which kinase?
(A) CDK1
(B) $CDK_4$
(C) p21
(D) Chk1

2009

**180.** Which class of molecules directly inhibits the activity of Cyclin-CDK complexes to slow or halt the cell cycle?
(A) Ubiquitin ligases
(B) Phosphatases like Cdc25 or PP2A
(C) Growth factor receptors
(D) Cyclin-dependent kinase inhibitors (CKIs, e.g., p21, p27)

2006

**181.** During which precise stage of mitosis do the sister chromatids separate and move toward opposite poles of the spindle?
(A) Prophase
(B) Metaphase
(C) Anaphase
(D) Telophase

2009

**182.** Crossing over, the exchange of genetic material between homologous chromosomes, occurs during which stage of meiosis?
(A) Prophase I
(B) Prophase II
(C) Metaphase I
(D) Anaphase I

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2010

**183.** The synaptonemal complex is a protein structure that strictly mediates which of the following processes?
(A) Pairing of homologous chromosomes (synapsis) during prophase I of meiosis
(B) Attachment of spindle microtubules to the chromosome kinetochore
(C) Separation of sister chromatids during anaphase II of meiosis
(D) Formation of the cleavage furrow during animal cell cytokinesis

2010

**184.** In animal cells, the mitotic spindle apparatus is primarily organized by which pair of structures?
(A) Centrosomes
(B) Centromeres
(C) Kinetochores
(D) Nucleoli

2025

**185.** Cytokinesis in plant cells differs fundamentally from that in animal cells due to the formation of a:
(A) Cleavage furrow driven by an actin-myosin contractile ring
(B) Septum forming exclusively from the plasma membrane invagination
(C) Spindle equator made solely of intermediate filaments
(D) Cell plate derived from Golgi vesicles fusing at the cell equator

2001

**186.** Match the mitotic/meiotic phase in List I with its defining event in List II:
List I: (Phase)
P. Metaphase (Mitosis)
Q. Anaphase I (Meiosis)
R. Diplotene (Meiosis I)
S. Telophase (Mitosis)
List II: (Defining Event)
1. Homologous chromosomes separate
2. Chiasmata become clearly visible
3. Chromosomes align at the equator
4. Nuclear envelope reforms
(A) P-3, Q-1, R-2, S-4
(B) P-1, Q-2, R-3, S-4
(C) P-3, Q-2, R-1, S-4
(D) P-2, Q-3, R-4, S-1

2003

**187.** What is the fundamental ploidy outcome of meiosis from a single diploid progenitor cell?
(A) Four genetically unique haploid cells
(B) Two identical diploid daughter cells
(C) Four identical diploid daughter cells
(D) Two genetically unique haploid cells

2021

**188.** Identify the INCORRECT statement regarding the kinetochore.
(A) It is a protein complex assembled on the centromeric region of the DNA.
(B) It acts as the primary attachment site for spindle microtubules during mitosis.
(C) It physically holds the two sister chromatids together along their entire length.
(D) It actively participates in the spindle assembly checkpoint signaling pathway.

2018

**189.** During which specific sub-stage of Prophase I does the actual process of crossing over between non-sister chromatids take place?
(A) Pachytene
(B) Leptotene
(C) Zygotene
(D) Diakinesis

2021

**190.** Which class of microtubules interacts with microtubules from the opposite pole to stabilize the spindle and push the poles apart during anaphase B?
(A) Kinetochore microtubules of the spindle
(B) Astral microtubules
(C) Centromeric microtubules of the spindle
(D) Polar (interpolar) microtubules

2024

**191.** Non-disjunction, the failure of chromosomes to separate properly, during Anaphase I of meiosis results in gametes with which chromosomal constitution?
(A) Two $(n+1)$ and two $(n-1)$
(B) All normal $(n)$
(C) Two normal $(n)$, one $(n+1)$, and one $(n-1)$
(D) All $(n+1)$

2012

**192.** Which enzyme is responsible for cleaving the cohesin rings that hold sister chromatids together, triggering the onset of anaphase?
(A) Securin
(B) Separase
(C) CDK1
(D) Caspase-3

2024

**193.** The concept of cellular totipotency refers to the ability of a single cell to:
(A) Divide continuously and indefinitely without entering cellular senescence
(B) Differentiate into any cell type of the adult organism, including extraembryonic tissues
(C) Form only blood-derived lineage cells within the marrow
(D) Shrink and form apoptotic bodies safely for disposal

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2002
**194.** Embryonic stem (ES) cells derived from the inner cell mass of a mammalian blastocyst are best classified as:
(A) Totipotent
(B) Multipotent
(C) Unipotent
(D) Pluripotent

2010
**195.** Adult stem cells, such as hematopoietic stem cells in the bone marrow, are typically described by which level of developmental potential?
(A) Multipotent
(B) Totipotent
(C) Pluripotent
(D) Nullipotent

2011
**196.** The deliberate conversion of a fully differentiated somatic cell back into a pluripotent stem cell (iPS cell) was famously achieved by introducing a specific set of:
(A) Lysosomal enzymes
(B) Ribosomal RNAs
(C) Transmembrane receptor tyrosine kinases of the plasma membrane
(D) Transcription factors (e.g., Oct4, Sox2, c-Myc, Klf4)

2016
**197.** Cellular differentiation is fundamentally driven by which underlying molecular mechanism?
(A) The differential expression of specific genes while maintaining a constant genome
(B) The loss or physical deletion of unneeded genes from the genome of the cell
(C) The continuous mutation of DNA sequences in specialized tissue types
(D) The varying speed of the cell cycle in different tissues

2016
**198.** Which plant tissue contains undifferentiated cells capable of active division and is largely responsible for continuous plant growth?
(A) Xylem
(B) Phloem
(C) Meristem
(D) Epidermis

2001
**199.** As a cell differentiates down a specific lineage, its developmental potential generally:
(A) Decreases
(B) Increases
(C) Remains constant
(D) Fluctuates randomly

2006
**200.** Identify the INCORRECT statement regarding stem cells.
(A) They are capable of selF-renewal through asymmetric cell division.
(B) They can produce progenitor cells that go on to differentiate.
(C) Their behavior is strictly regulated by microenvironmental signals (the stem cell niche).
(D) They exhibit a highly restricted and rapid rate of apoptosis to maintain numbers.

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2022
**201.** Following are some statements concerning cellular growth and differentiation:
I. All differentiated plant cells irreversibly lose totipotency.
II. Pluripotent cells can form all three germ layers: ectoderm, mesoderm, and endoderm.
III. Unipotent stem cells can only differentiate into one specific cell type.
Which of the above statement(s) is/are INCORRECT?
(A) I only
(B) I and II
(C) II and III
(D) III only

2024
**202.** Which type of cellular growth involves an increase in tissue size due strictly to an increase in the size of individual cells, without cell division?
(A) Hyperplasia
(B) Hypertrophy
(C) Dysplasia
(D) Neoplasia

2018
**203.** Epigenetic modifications play a crucial role in cellular differentiation. Which of the following is a classic example of an epigenetic modification?
(A) DNA methylation of cytosine residues in promoter regions
(B) Deletion of a nucleotide base
(C) Alternative splicing of mRNA
(D) Frameshift mutation in a structural gene of the cell

2009
**204.** During differentiation, cells often respond to external signaling molecules secreted by

neighboring cells, known as morphogens. Morphogens function primarily by:
(A) Entering the nucleus and directly cutting DNA
(B) Forming concentration gradients that trigger different developmental programs
(C) Dissolving the plasma membrane to fuse cells together
(D) Acting as a structural component of the extracellular matrix of the cell

2012
**205.** Apoptosis is formally defined as which of the following?
(A) Pathological cell death caused by severe acute injury or infection
(B) Programmed cell death characterized by rapid cellular swelling and membrane rupture
(C) Programmed, energy-dependent cellular suicide characterized by DNA fragmentation and membrane blebbing
(D) The aging and eventual death of a cell due to telomere shortening

2024
**206.** Which morphological feature strongly distinguishes apoptosis from necrosis?
(A) Extensive inflammatory response in the surrounding tissue
(B) Rupture of the plasma membrane spilling contents out
(C) Swelling of the cell and internal organelles
(D) Shrinkage of the cell and packaging into intact apoptotic bodies

2023
**207.** The central executioners of the apoptotic pathway are a family of cysteine proteases known as:
(A) Cathepsins
(B) Calpains
(C) Matrix metalloproteinases
(D) Caspases

2018
**208.** The intrinsic (mitochondrial) pathway of apoptosis is primarily triggered by:
(A) The binding of a death ligand (e.g., FasL) to a death receptor on the surface
(B) Internal cellular stress, such as severe DNA damage or oxidative stress
(C) The physical crushing of the cell membrane
(D) Depletion of extracellular sodium

2023
**209.** In the intrinsic apoptotic pathway, which critical molecule is released from the mitochondrial intermembrane space into the cytosol to activate the apoptosome?
(A) Cytochrome c
(B) ATP
(C) NADH
(D) Calcium ions

2009
**210.** Which family of proteins critically regulates the permeability of the outer mitochondrial membrane during the intrinsic apoptotic pathway?
(A) The Caspase family
(B) The Cyclin family
(C) The SNARE family
(D) The BCl-2 family

2023
**211.** Following are some statements regarding the regulators of apoptosis:
I. Bax and Bak are pro-apoptotic members of the BCl-2 family.
II. BCl-2 and BCl-xL are anti-apoptotic proteins.
III. The apoptosome strictly activates Caspase-8.
IV. Extrinsic apoptosis involves Death-Inducing Signaling Complex (DISC).
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I and II
(C) II, III and IV
(D) I, III and IV

2022
**212.** The extrinsic pathway of apoptosis is initiated by the binding of a ligand to a death receptor. Which of the following is a classic example of a death receptor?
(A) Insulin receptor
(B) Epidermal growth factor receptor (EGFR)
(C) Estrogen receptor
(D) Fas receptor (CD95)

2017
**213.** Caspase-8 and Caspase-9 are categorized based on their role in the apoptotic cascade as:
(A) Initiator caspases
(B) Executioner caspases
(C) Inflammatory caspases
(D) Scaffold proteins

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2017

**214.** Which specific biochemical event provides the distinct "ladder" pattern of DNA seen during gel electrophoresis of apoptotic cells?
(A) Cleavage of DNA between nucleosomes by Caspase-Activated DNase (CAD)
(B) Random degradation of DNA by lysosomal acid DNase within the organelle
(C) Incomplete DNA replication blocking S phase
(D) The complete dissolution of histone proteins

2002

**215.** During apoptosis, which lipid flips from the inner leaflet to the outer leaflet of the plasma membrane to act as an "eat-me" signal for macrophages?
(A) Phosphatidylcholine
(B) Sphingomyelin
(C) Phosphatidylserine
(D) Cholesterol

2019

**216.** Identify the INCORRECT statement comparing apoptosis and necrosis.
(A) Apoptosis requires ATP; necrosis does not.
(B) Necrosis elicits an inflammatory response; apoptosis typically does not in tissues.
(C) Apoptosis usually affects single cells; necrosis often affects contiguous groups of cells.
(D) DNA cleavage in necrosis is highly specific and internucleosomal, unlike apoptosis.

2004

**217.** In which type of cellular signaling does a cell secrete a signaling molecule that binds to receptors on its own surface?
(A) Autocrine signaling
(B) Endocrine signaling
(C) Paracrine signaling
(D) Juxtacrine signaling

2007

**218.** Endocrine signaling is characterized by signaling molecules (hormones) that:
(A) Act exclusively on immediately adjacent cells
(B) Are transferred directly through cell gap junctions
(C) Are attached to the membrane of the signaling cell
(D) Travel through the bloodstream to act on distant target cells

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2015

**219.** Synaptic transmission between a motor neuron and a muscle cell is a highly specialized form of which signaling type?
(A) Paracrine signaling
(B) Endocrine signaling
(C) Autocrine signaling
(D) Intracrine signaling

2001

**220.** The binding kinetics of a ligand to its receptor are often described by the dissociation constant (Kd). A lower Kd value strictly indicates:
(A) A lower affinity of the receptor for the ligand
(B) A higher concentration of ligand is required to achieve binding
(C) The receptor is located intracellularly
(D) A higher affinity of the receptor for the ligand

2005

**221.** Following are some statements regarding cell signaling principles:
I. Hydrophilic ligands typically bind to intracellular receptors.
II. A single signaling molecule can trigger different responses in different cell types.
III. Agonists are molecules that bind to a receptor and inhibit its natural response.
IV. Gap junctions allow direct juxtacrine-like transfer of small molecules.
V. Signal amplification is a common feature of signal transduction pathways.
Which of the above statement(s) is/are INCORRECT?
(A) I and III
(B) II and V
(C) I, III and IV
(D) III, IV and V

2006

**222.** Which process ensures that a target cell does not remain perpetually stimulated by a signaling molecule?
(A) Signal amplification
(B) Receptor upregulation
(C) Receptor desensitization or downtime
(D) Positive feedback looping

2012

**223.** What is the defining feature of juxtacrine signaling?
(A) It utilizes the systemic lymphatic system for long-distance transport.
(B) It relies on volatile gases acting as primary signaling molecules.

(C) It occurs only during programmed cell death or apoptosis.
(D) It requires direct physical contact between the signaling cell and the target cell.

2014
**224.** A molecule that binds to a receptor but fails to activate it, thereby blocking the endogenous ligand from binding, is known as a/an:
(A) Antagonist
(B) Full agonist
(C) Partial agonist
(D) Secondary messenger

2024
**225.** Nitric oxide (NO) is a unique signaling molecule because it:
(A) Cannot cross the cell membrane without a specialized protein transporter.
(B) Is a gas that diffuses directly across membranes to activate intracellular enzymes.
(C) Binds exclusively to G-protein coupled receptors on the plasma membrane.
(D) Functions as a long-lived endocrine hormone.

2019
**226.** In signal transduction, "cross-talk" refers to:
(A) The synthesis of two different ligands by the same cell for export.
(B) The rapid degradation of a receptor by intracellular lysosomes.
(C) The physical exchange of receptors between neighboring cell membranes.
(D) The interaction and integration of different signaling pathways within a cell.

2008
**227.** Which feature is universally essential for a molecule to act as a target cell receptor?
(A) It must possess a specific ligand-binding site and undergo a conformational change upon binding.
(B) It must be an integral membrane protein spanning the lipid bilayer exactly seven times.
(C) It must have intrinsic protein kinase activity.
(D) It must be located strictly in the nucleus.

2002
**228.** What term describes the phenomenon where successive steps in a signaling pathway recruit or activate increasingly larger numbers of molecules?
(A) Signal transduction processes
(B) Signal termination events
(C) Signal amplification (cascade)
(D) Signal attenuation methods

2013
**229.** G-protein coupled receptors (GPCRs) share a highly conserved structural feature, which is:
(A) They exist as intrinsic ion channels.
(B) They possess exactly one transmembrane alpha-helix.
(C) They span the plasma membrane seven times (7-TM domain).
(D) They possess intracellular domains with intrinsic tyrosine kinase activity.

2019
**230.** Upon ligand binding to a GPCR, what is the immediate molecular event that activates the associated heterotrimeric G-protein?
(A) The alpha subunit hydrolyzes GTP to GDP.
(B) The beta and gamma subunits degrade the alpha subunit.
(C) The G-protein physically detaches from the inner membrane leaf.
(D) The alpha subunit exchanges its bound GDP for GTP.

2007
**231.** Receptor Tyrosine Kinases (RTKs) are typically activated through which mechanism following ligand binding?
(A) Receptor dimerization and subsequent auto-cross-phosphorylation
(B) Immediate enzymatic shedding of the extracellular ligand-binding domain
(C) Binding to an intracellular G-protein for activation
(D) Opening of a central pore to allow rapid sodium influx

2003
**232.** The Ras protein, frequently mutated in human cancers, is activated in downstream RTK signaling. Ras belongs to which class of proteins?
(A) Heterotrimeric G-proteins
(B) Protein phosphatases
(C) Transcription factors
(D) Small monomeric GTPases

2024
**233.** The MAP kinase (MAPK) signaling cascade follows which general three-tiered sequential activation order?
(A) MAPK → MAPKK → MAPKKK
(B) MAPKKK → MAPKK → MAPK
(C) Raf → Ras → MEK
(D) ERK → MEK → Raf

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2019
**234.** Identify the INCORRECT statement regarding GPCR signaling.
(A) They constitute the largest family of cell-surface receptors.
(B) The activated alpha subunit dissociates from the beta-gamma complex.
(C) GPCRs directly phosphorylate target proteins in the cytosol.
(D) The alpha subunit has intrinsic GTPase activity to terminate the signal.

2001
**235.** Which specific domain on intracellular adaptor proteins (like Grb2) recognizes and binds to the phosphorylated tyrosines on activated RTKs?
(A) $SH_2$ domain
(B) PH domain
(C) KDEL sequence
(D) Helix-turn-helix motif

2012
**236.** Following two statements, one labeled as Assertion (A) and the other as Reason (R). Select the correct answer using the codes given below.
**Assertion (A):** Cholera toxin causes severe watery diarrhea by irreversibly activating the Gs-alpha subunit in intestinal cells.
**Reason (R):** It ADP-ribosylates the Gs-alpha subunit, destroying its intrinsic GTPase activity, leading to continuous cAMP production.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2016
**237.** The insulin receptor is a classic example of which type of cell surface receptor?
(A) Receptor Tyrosine Kinase (RTK)
(B) G-protein coupled receptor
(C) Ligand-gated ion channel
(D) Integrin receptor

2002
**238.** In the context of RTK signaling, what is the primary role of scaffolding proteins?
(A) They degrade the primary ligand to terminate signaling in the matrix.
(B) They actively transport the receptor to the nuclear compartment.
(C) They act as competitive inhibitors for ATP binding.
(D) They bind multiple components of a kinase cascade to ensure efficiency and specificity.

2008
**239.** Which enzyme is responsible for turning off the active MAPK signal by removing phosphate groups from the kinases?
(A) Protein phosphatase
(B) Adenylate cyclase
(C) Phospholipase C
(D) Guanyl nucleotide exchange factor (GEF)

2018
**240.** The interaction between the Gq-alpha subunit and its primary effector enzyme directly leads to the generation of which secondary messengers?
(A) cAMP and cGMP
(B) Calcium and Nitric Oxide
(C) Ras and Raf
(D) IP3 and DAG

2009
**241.** Cyclic AMP (cAMP) is generated from ATP by the action of which membrane-bound enzyme?
(A) Phosphodiesterase
(B) Adenylate cyclase
(C) Phospholipase C
(D) Protein Kinase A

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2018
**242.** The primary intracellular target that is activated by elevated levels of cAMP is:
(A) Protein Kinase C (PKC)
(B) Protein Kinase A (PKA)
(C) Calmodulin
(D) IP3 receptor

2011
**243.** How is the cAMP signal rapidly terminated within the cell?
(A) Re-conversion of cAMP to ATP by adenylate cyclase
(B) Hydrolysis of cAMP to 5'-AMP by phosphodiesterase
(C) Export of cAMP out of the cell via ABC transporters
(D) Direct degradation of cAMP by lysosomes

2023
**244.** Phospholipase C (PLC) cleaves the membrane phospholipid PIP2 into which two second messengers?
(A) Diacylglycerol (DAG) and Inositol 1,4,5-trisphosphate (IP3)
(B) cAMP and cGMP
(C) Arachidonic acid and assorted prostaglandin

metabolites
(D) Calcium and calmodulin

2017
**245.** Following its generation by PLC, what is the specific biological role of water-soluble IP3?
(A) It activates Protein Kinase C at the plasma membrane through lipid interaction.
(B) It binds to receptors on the smooth ER, causing the release of stored $Ca^{2+}$ into the cytosol.
(C) It directly enters the nucleus to alter gene transcription in response to signals.
(D) It inhibits adenylate cyclase.

2019
**246.** Match the second messenger in List I with its specific direct target or property in List II:
List I: (Second Messenger)
P. cAMP
Q. cGMP
R. DAG
S. Calcium ($Ca^{2+}$)
List II: (Target/Property)
1. Activates Protein Kinase G (PKG)
2. Binds specifically to Calmodulin
3. Activates Protein Kinase A (PKA)
4. Remains embedded in the plasma membrane to activate PKC
(A) P-3, Q-1, R-4, S-2
(B) P-1, Q-3, R-2, S-4
(C) P-3, Q-4, R-1, S-2
(D) P-2, Q-1, R-4, S-3

2005
**247.** The second messenger cGMP is critical in which highly specialized physiological process?
(A) Visual phototransduction cascade in rod cells of the retina
(B) Uterine contraction during childbirth
(C) The synthesis of thyroid hormone
(D) The initial breakdown of glycogen stores in skeletal muscle

2018
**248.** Diacylglycerol (DAG) activates Protein Kinase C (PKC), but full activation of PKC typically also requires the presence of which other ion?
(A) $Na^+$
(B) $K^+$
(C) $Ca^{2+}$
(D) $MG_2^+$

2003
**249.** Identify the INCORRECT statement regarding intracellular calcium as a second messenger.
(A) It is actively pumped out of the cytosol to maintain the resting state.
(B) It can trigger massive exocytosis, such as neurotransmitter release.
(C) It heavily influences muscle contraction via troponin C.
(D) The resting cytosolic concentration of $Ca^{2+}$ is kept extremely high.

2011
**250.** Calmodulin is a ubiquitous calcium-binding protein. Upon binding calcium, what does the $Ca^{2+}$/Calmodulin complex do?
(A) It acts as a protease to destroy damaged receptors within the cell.
(B) It wraps around and activates various target proteins, such as CaM-kinases.
(C) It pumps calcium back into the endoplasmic reticulum using ATP energy.
(D) It binds directly to DNA to initiate transcription.

2007
**251.** Which gas diffuses into target cells to activate soluble guanylate cyclase, leading to cGMP production and smooth muscle relaxation?
(A) Atmospheric carbon dioxide ($CO_2$)
(B) Nitric oxide (NO)
(C) Stable carbon monoxide (CO)
(D) Medical nitrous oxide ($N_2O$)

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2019
**252.** The amplification effect of a second messenger cascade ensures that:
(A) A single ligand molecule can ultimately activate thousands of effector proteins.
(B) The response strictly remains localized to the primary receptor site.
(C) The cell rapidly undergoes apoptosis after receiving the initial signal.
(D) The second messenger is immediately exported from the cytosolic space.

2024
**253.** Intracellular receptors are typically located in the cytosol or nucleus. What defining physical property must their ligands possess?
(A) Small size and hydrophobicity to cross the plasma membrane
(B) High molecular weight and strong polarity for solubility
(C) Highly charged and water-soluble
(D) Exclusively gaseous state

2021

**254.** The steroid receptor superfamily includes receptors for which of the following hormones?
(A) Insulin and Glucagon
(B) Cortisol, Estrogen, and Testosterone
(C) Epinephrine and Norepinephrine
(D) Growth Hormone and Prolactin

2024

**255.** Upon binding its hydrophobic ligand, an intracellular steroid receptor typically acts directly as a:
(A) Ligand-dependent transcription factor
(B) G-protein alpha subunit
(C) Protein kinase
(D) Ribosomal translation factor

2005

**256.** Which structural domain is highly conserved across the steroid receptor superfamily and is essential for recognizing specific DNA sequences?
(A) Transmembrane domain
(B) $SH_2$ domain
(C) Zinc-finger DNA-binding domain
(D) Pleckstrin homology (PH) signaling domain

2001

**257.** In the absence of a ligand, glucocorticoid receptors are typically held in an inactive state in the cytosol bound to which chaperone protein?
(A) Hsp90 (Heat shock protein 90)
(B) Clathrin
(C) BiP
(D) Ubiquitin

2003

**258.** What happens immediately after a steroid hormone binds to its specific cytosolic receptor?
(A) The receptor undergoes rapid proteolytic degradation in the cytosol.
(B) The receptor undergoes a conformational change, releases chaperones, and translocates to the nucleus.
(C) The receptor is embedded into the plasma membrane.
(D) The hormone is enzymatically cleaved by the receptor.

2008

**259.** Hormone Response Elements (HREs) are best defined as:
(A) Specific sequences in the mRNA that regulate translation and protein synthesis.
(B) Specific DNA sequences in the promoter/enhancer regions of target genes where the receptor binds
(C) Transmembrane channels that import steroid hormones
(D) Cytosolic enzymes that degrade excess hormones

2004

**260.** Unlike steroid hormones, thyroid hormones ($T_3$/$T_4$) act via receptors that are primarily located where, even in the unliganded state?
(A) At the plasma membrane
(B) In the lysosome
(C) Bound to DNA in the nucleus, often repressing transcription
(D) Free-floating in the cytosolic compartment

2017

**261.** Peptide hormones (e.g., insulin, oxytocin) cannot utilize intracellular receptors. Why?
(A) They are large and/or hydrophilic, thus incapable of diffusing across the lipid bilayer.
(B) They are instantly delegated by cytosolic proteases.
(C) They lack the necessary zinc-finger motifs.
(D) They do not regulate gene transcription under any biological circumstances.

2001

**262.** Identify the INCORRECT statement regarding the action of intracellular receptors.
(A) Their primary mechanism involves altering gene expression.
(B) The physiological response they induce is typically much faster (milliseconds) than that of ion channel receptors.
(C) The ligand-receptor complex can recruit co-activators or co-repressors to chromatin for regulation.
(D) Vitamin D and Retinoic Acid also signal via this receptor superfamily.

2013

**263.** The primary functional response of target gene transcription regulation by steroid hormones is strictly classified as:
(A) Genomic action
(B) Non-genomic action
(C) Autocrine regulation
(D) Contact-dependent inhibition

2013

**264.** Selective Estrogen Receptor Modulators (SERMs), like tamoxifen, exhibit which unique pharmacological property?
(A) They strictly degrade estrogen.
(B) They convert testosterone into estrogen.
(C) They act as an antagonist in some tissues

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(e.g., breast) but an agonist in others (e.g., bone).
(D) They prevent estrogen from entering through the cell membrane barrier.

2021
**265.** The specialized junction between a neuron and its target cell (another neuron or muscle) is known as the:
(A) Plasmodesma
(B) Synapse
(C) Tight junction
(D) Desmosome

2004
**266.** Neurotransmitter release at the presynaptic terminal is strictly triggered by the influx of which ion through voltage-gated channels?
(A) $Na^+$
(B) $K^+$
(C) $Cl^-$
(D) $Ca^{2+}$

2024
**267.** Ligand-gated ion channels at the postsynaptic membrane operate by:
(A) Directly opening an ion pore upon binding a specific neurotransmitter
(B) Activating internal G-proteins to produce cAMP
(C) Physically transporting the neurotransmitter into the postsynaptic cell via channels
(D) Phosphorylating intracellular target proteins

2013
**268.** The nicotinic acetylcholine receptor at the neuromuscular junction is a classic example of a:
(A) G-protein coupled receptor
(B) Tyrosine kinase receptor
(C) Ligand-gated cation channel
(D) Mechanically gated channel

2019
**269.** Gap junctions in animal cells provide which crucial functional capability?
(A) They seal the space between adjacent cells to prevent fluid leakage across layers.
(B) They anchor cells strongly to the basal lamina.
(C) They allow direct electrical and metabolic coupling by passing small ions and molecules between cells.
(D) They transport large organelles between neighboring cells.

2007
**270.** The structural channel of a gap junction is called a connexon, which is composed of how many connexin protein subunits?
(A) 2
(B) 4
(C) 6
(D) 8

2025
**271.** Plasmodesmata are intercellular junctions functionally analogous to gap junctions, but they are found exclusively in:
(A) Cardiac muscle cells
(B) Fungal hyphae
(C) Plant cells
(D) Epithelial layers of the gut

2018
**272.** A structurally unique feature of a plasmodesma is the presence of the desmotubule. The desmotubule is a direct continuation of which organelle between two adjacent plant cells?
(A) Smooth Endoplasmic Reticulum
(B) Golgi Apparatus stacks
(C) Mitochondrion
(D) Vacuole

2025
**273.** Desmosomes (maculae adherentes) function primarily to:
(A) Prevent the diffusion of membrane proteins within the lipid bilayer.
(B) Provide strong mechanical adhesion between adjacent cells to resist shearing forces
(C) Allow the rapid transmission of electrical impulses across the tissue.
(D) Actively pump nutrients between cells.

2007
**274.** The strong mechanical integrity provided by desmosomes is achieved by anchoring their intracellular plaques to which specific cytoskeletal network?
(A) Microtubules
(B) Actin microfilaments
(C) Intermediate filaments (e.g., keratin)
(D) Myosin thick motor filaments

2009
**275.** Identify the INCORRECT statement regarding synaptic transmission.
(A) It relies on the exocytosis of synaptic vesicles.
(B) Neurotransmitters can cause either excitatory or inhibitory potentials in the target.

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(C) The process strictly operates in a bidirectional manner across the same chemical synapse.
(D) Neurotransmitters must be cleared from the cleft to terminate the signal.

2002
**276.** Tight junctions (zonulae occludentes) are critical in epithelial layers mainly because they:
(A) Allow unrestricted flow of ions.
(B) Prevent the leakage of solutes and water between the cells (paracellular pathway)
(C) Anchor the cells to the underlying extracellular matrix proteins.
(D) Contain pores for mRNA transfer.

2016
**277.** The first step in most subcellular analytical techniques requires breaking open the cell membrane while preserving organelle integrity. This process is termed:
(A) Cell lysis or homogenization
(B) Differential centrifugation
(C) Chemical fixation
(D) Flow cytometry

2004
**278.** Which of the following is a gentle, mechanical method of cell lysis that prevents thermal denaturation of proteins?
(A) Dounce homogenization
(B) Boiling in SDS buffer
(C) Exposure to highly basic solutions
(D) Autoclaving

2002
**279.** Differential centrifugation separates cellular components strictly based on which physical properties?
(A) Surface charge and hydrophobicity
(B) Light scattering and fluorescence
(C) Specific binding affinities to antibodies
(D) Size and density

2014
**280.** During a standard differential centrifugation of a tissue homogenate, which cellular component will typically pellet first at the lowest speed (e.g., 600 x g)?
(A) Ribosomes
(B) Microsomes (ER fragments)
(C) Nuclei and unbroken cells
(D) Mitochondria and lysosomes

2003
**281.** Following the pelleting of nuclei, a subsequent centrifugation at a moderate speed (~15,000 x g) will primarily pellet which fraction?
(A) Soluble cytosolic proteins and small metabolites
(B) Intact ribosomes
(C) Small vesicles of the ER
(D) Mitochondria, chloroplasts, and peroxisomes

2014
**282.** To separate organelles that have very similar sizes but different densities (such as lysosomes and peroxisomes), which advanced centrifugation technique is used?
(A) Isopycnic (density-gradient) ultracentrifugation
(B) Differential precipitation
(C) Zonal electrophoresis
(D) Gel filtration chromatography

2021
**283.** In isopycnic ultracentrifugation, an organelle will migrate through a sucrose or Percoll gradient until it reaches:
(A) The bottom of the tube
(B) A position where its density perfectly matches the density of the surrounding gradient
(C) The meniscus at the top
(D) A specific position where the net centrifugal force becomes zero

2011
**284.** Flow cytometry is a powerful analytical technique used to:
(A) Rapidly analyze physical and chemical characteristics of single cells as they flow in a fluid stream through a laser
(B) Directly sequence genomic DNA
(C) Cut ultra-thin slices of fixed tissue for electron microscopy imaging
(D) Measure the exact mass of individual proteins

2002
**285.** In Flow Cytometry, the Forward Scatter (FSC) detector primarily provides information regarding the cell's:
(A) Internal complexity or granularity
(B) Total DNA content
(C) Cell surface charge
(D) Relative size or volume

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2011
**286.** Conversely, the Side Scatter (SSC) detector in a flow cytometer measures light scattered at a 90-degree angle and correlates with the cell's:
(A) Internal complexity and granularity
(B) Relative size
(C) Rate of mitosis
(D) Overall buoyancy

2025
**287.** FACS stands for Fluorescence-Activated Cell Sorting. What specific capability does FACS add to standard flow cytometry?
(A) The ability to physically separate and collect distinct cell subpopulations based on their fluorescent signals
(B) The ability to image the cells in three dimensions
(C) The ability to lyse the cells instantly for high-yield DNA extraction
(D) The ability to grow cells rapidly in suspension

2022
**288.** Which fluorescent probe is commonly used in flow cytometry to quantitatively measure the DNA content of cells for cell cycle analysis?
(A) Propidium Iodide (PI)
(B) Green Fluorescent Protein (GFP)
(C) Fluorescein Isothiocyanate (FITC)
(D) Coomassie Brilliant Blue

2006
**289.** Phase-contrast microscopy dramatically improves the visibility of live, unstained cells by exploiting which optical phenomenon?
(A) The emission of longer wavelength light after UV excitation
(B) The complete polarization of all incoming light
(C) The absorption of specific wavelengths of light by naturally colored intracellular pigments
(D) Differences in refractive index and thickness within cellular structures converted into differences in light intensity

2004
**290.** The fundamental principle of fluorescence microscopy involves:
(A) Passing a beam of high-energy electrons through the sample
(B) Illuminating the specimen with light of a specific excitation wavelength and capturing the emitted light of a longer wavelength
(C) Using heavy metal stains to deflect photons from the light source
(D) Scanning a physical probe over the surface of the cell

2016
**291.** Which specific optical component in a fluorescence microscope ensures that only the emitted light from the fluorophore reaches the eyepiece/camera, while blocking the excitation light?
(A) Phase plate
(B) Dichroic mirror and emission filter
(C) Microscope condenser lens
(D) Microscope objective lens

2006
**292.** Confocal microscopy provides a significantly sharper, three-dimensional image of thick specimens compared to widefield fluorescence microscopy by utilizing a:
(A) Vacuum chamber
(B) Pinhole aperture to eliminate out-oF-focus light from planes above and below the focal plane
(C) Much brighter halogen lamp
(D) Specialized electron-sensitive detector

2019
**293.** Transmission Electron Microscopy (TEM) can achieve vastly superior resolution compared to light microscopy primarily because:
(A) The wavelength of an electron beam is thousands of times shorter than the wavelength of visible light.
(B) The lenses used in TEM are made of highly polished diamond or specialized sapphire.
(C) TEM operates in a liquid medium rather than air.
(D) Electrons possess high mass and naturally colorize the specimen during imaging.

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2001
**294.** In Transmission Electron Microscopy (TEM), biological specimens are typically stained with salts of heavy metals (like osmium tetroxide or uranyl acetate) in order to:
(A) Keep the cells alive in the vacuum chamber.
(B) Scatter electrons to create contrast in the final image.
(C) Prevent the specimen from shrinking.
(D) Make the specimen emit fluorescent light for detection.

2013
**295.** Scanning Electron Microscopy (SEM) is optimally designed for which specific application?
(A) Observing the real-time movement of

organelles in a living cell
(B) Visualizing the fine internal cross-sectional ultrastructure of the cell nucleus
(C) Generating highly detailed 3D images of the exterior surface topology of a specimen
(D) Determining the precise molecular weight of a protein complex

2024

**296.** Which step is essential for sample preparation in SEM but generally not in TEM?
(A) Embedding the sample in hard resin.
(B) Cutting the sample into ultra-thin sections using an automated ultramicrotome device.
(C) Treating the sample with a primary antibody.
(D) Sputter-coating the exterior surface of the specimen with a thin layer of conductive metal, such as gold

2008

**297.** The theoretical limit of resolution for a standard light microscope is dictated by Abbe's diffraction limit, which is approximately:
(A) 0.1 nm
(B) 2 nm
(C) 200 nm
(D) 50 micrometers

2024

**298.** Green Fluorescent Protein (GFP), widely used in live-cell imaging to tag cellular proteins, was originally isolated from which organism?
(A) Escherichia coli
(B) Saccharomyces cerevisiae
(C) The jellyfish Aequorea victoria
(D) The firefly Photinus pyralis

2014

**299.** Differential Interference Contrast (DIC) microscopy, like phase-contrast, enhances the contrast of transparent specimens but produces an image with a characteristic:
(A) Pseudo-3D, shadow-cast appearance
(B) Bright glowing background
(C) Completely black and white binary output
(D) Neon color map indicating chemical composition

2011

**300.** The application of antibodies conjugated to a fluorophore to specifically detect and localize a particular protein within a fixed cell under a microscope is termed:
(A) Western blotting
(B) Immunofluorescence
(C) Chromatin immunoprecipitation
(D) Differential centrifugation

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# ANSWERS

**1.** (A) RNA world hypothesis
The RNA world hypothesis proposes that early life forms utilized ribonucleic acid as both a genetic repository and a biological catalyst. Unlike DNA, RNA can fold into complex shapes to catalyze chemical reactions, similar to modern enzymes. This theory suggests that RNA preceded DNA and proteins in the evolutionary timeline of life on Earth.

**2.** (C) Ultraviolet radiation and lightning
Primitive Earth lacked a protective ozone layer, allowing intense ultraviolet radiation from the sun to reach the surface. Coupled with frequent volcanic activity and electrical discharges from lightning, these high-energy sources provided the necessary power to drive the synthesis of complex organic molecules from simple atmospheric gases like methane, ammonia, hydrogen, and water vapor.

**3.** (A) Possession of circular DNA and 70S ribosomes
Mitochondria exhibit structural and genetic features that strongly resemble prokaryotic cells rather than eukaryotic ones. They possess their own circular DNA molecules and 70S ribosomes, which are characteristic of bacteria. These traits support the theory that mitochondria originated from an ancestral endosymbiotic relationship where a host cell engulfed a free-living aerobic bacterium.

**4.** (D) RNA is chemically more stable than DNA, making it an ideal long-term storage molecule.
Deoxyribonucleic acid is chemically more stable than ribonucleic acid because it lacks the 2'-hydroxyl group on its pentose sugar, which makes RNA prone to hydrolysis. Furthermore, DNA's double-stranded structure provides extra protection for genetic information. Therefore, DNA replaced RNA as the primary long-term storage molecule for genetic information in all complex organisms.

**5.** (B) Reducing, composed mainly of methane, ammonia, hydrogen, and water vapor
The Oparin-Haldane model posits that life emerged from a reducing atmosphere that lacked free molecular oxygen. This environment was dominated by gases like methane, ammonia, and hydrogen. Under these conditions, energy sources could facilitate the formation of organic monomers, which eventually accumulated in the primitive oceans, creating a prebiotic soup necessary for early life.

**6.** (D) It assumed a highly reducing atmosphere, which early Earth may not have had.
While the Miller-Urey experiment successfully produced amino acids, modern geochemical evidence suggests that the early atmosphere was likely neutral rather than highly reducing. It probably contained more carbon dioxide and nitrogen than the methane and ammonia used in the simulation. This discrepancy challenges whether the specific chemical pathways demonstrated in the lab occurred on Earth.

**7.** (D) Free-living photosynthetic cyanobacteria
Modern chloroplasts share significant structural and genetic similarities with cyanobacteria, including a double-membrane system and similar photosynthetic machinery. According to endosymbiotic theory, an ancestral eukaryotic cell engulfed a photosynthetic cyanobacterium. Instead of being digested, the bacterium survived and entered a symbiotic relationship, eventually evolving into the specialized plastid responsible for photosynthesis in plants and algae.

**8.** (A) Both A and R are true and R is the correct explanation of A.
Mitochondria and chloroplasts are termed semi-autonomous because they possess their own circular genomes and ribosomes to produce some internal proteins. However, they are not fully independent since they rely on the host cell's nucleus for many essential proteins and enzymes. This dual genetic origin confirms their status as semi-autonomous units within the larger eukaryotic cell.

**9.** (B) Droplets of organic polymers that form spontaneously in a liquid phase
Coacervates are spherical droplets formed by the spontaneous aggregation of organic macromolecules through hydrophobic

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interactions in an aqueous medium. Proposed by Oparin, these structures represent a crucial step in chemical evolution. They demonstrate how early organic molecules could have achieved compartmentalization, allowing for a primitive metabolism and internal environment distinct from the surrounding prebiotic soup.

**10.** (B) Pasteur's swan-neck flask experiment
Louis Pasteur used swan-neck flasks to demonstrate that microorganisms only grow in a nutrient broth when exposed to airborne particles. By preventing dust from reaching the broth while allowing air to enter, he showed that life does not arise spontaneously from non-living matter. This experiment effectively discredited the theory of spontaneous generation and established the principle of biogenesis.

**11.** (A) Deoxyribose and the absence of a 2'-OH group make DNA less prone to hydrolysis.
The chemical evolution from RNA to DNA was driven by the need for greater genetic stability. The absence of the 2'-hydroxyl group in deoxyribose makes the DNA backbone much more resistant to alkaline hydrolysis compared to RNA. This increased stability allows DNA to serve as a reliable long-term repository for the complex genetic instructions required by organisms.

**12.** (C) Engulfment of a photosynthetic eukaryote by a heterotrophic eukaryote
Secondary endosymbiosis occurs when a heterotrophic eukaryotic cell engulfs another eukaryotic cell that already contains a primary plastid, such as a red or green alga. This process leads to the formation of complex plastids surrounded by three or four membranes. It explains the diversity of photosynthetic lineages found in various groups, including diatoms, brown algae, and euglenids.

**13.** (D) Peptidoglycan-based cell wall
Peptidoglycan is a unique polymer consisting of sugars and amino acids that forms a mesh-like layer outside the plasma membrane of most bacteria. This structural component provides rigidity and protection to the prokaryotic cell wall. Eukaryotic cells, such as those in plants or fungi, utilize different materials like cellulose or chitin, and never contain peptidoglycan in their structures.

**14.** (C) It allows for the spatial separation and optimization of competing biochemical pathways.
Eukaryotic cells utilize membrane-bound organelles to create distinct internal environments. This compartmentalization allows various biochemical processes, which might otherwise interfere with one another, to occur simultaneously and efficiently. By concentrating specific enzymes and substrates within organelles like mitochondria or lysosomes, the cell can precisely regulate its metabolism and respond more effectively to changing physiological needs.

**15.** (B) Plant cells contain a large central vacuole; animal cells typically have small or no vacuoles.
A defining difference between plant and animal cells is the presence of a large central vacuole in plants. This organelle maintains turgor pressure against the cell wall, providing structural support. While animal cells may possess small, temporary vacuoles for transport or storage, they lack the massive, permanent central compartment that characterizes the mature eukaryotic plant cell architecture.

**16.** (D) Flagella composed of the protein flagellin
Prokaryotic flagella are simple, rotating appendages made primarily of the protein flagellin. They function like a propeller to provide motility. In contrast, eukaryotic flagella are complex structures composed of tubulin microfilaments arranged in a 9+2 pattern and move in a whip-like fashion. This fundamental difference in protein composition and movement mechanism distinguishes the two cellular domains.

**17.** (A) Prokaryotic genomes typically consist of a single circular chromosome located in the nucleoid.
Prokaryotic organisms generally organize their genetic information into a single, circular DNA molecule that is not enclosed within a nuclear envelope. This region is known as the nucleoid. Unlike eukaryotes, which have multiple linear chromosomes associated with histone proteins, prokaryotic DNA is typically much smaller and lacks the complex nucleosome structure found in more advanced eukaryotic cells.

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**18.** (C) They carry accessory genes that may provide selective advantages, such as antibiotic resistance.
Plasmids are small, extrachromosomal circular DNA molecules found in many bacteria. While they do not carry genes essential for basic survival, they often contain accessory genetic information that provides significant benefits. Common examples include genes for antibiotic resistance, toxin production, or the ability to metabolize unusual substances, allowing the bacteria to survive in diverse or hostile environments.

**19.** (A) Gram-negative cells feature a thin peptidoglycan layer situated between an inner and outer membrane.
The structural difference between bacterial cell envelopes is revealed by Gram staining. Gram-negative bacteria possess a complex envelope with an inner cytoplasmic membrane, a thin peptidoglycan layer, and a specialized outer membrane containing lipopolysaccharides. Gram-positive bacteria, however, lack the outer membrane but have a much thicker peptidoglycan layer, which allows them to retain the primary stain.

**20.** (D) The inner mitochondrial membrane
In prokaryotes, the machinery for aerobic respiration and the electron transport chain is located directly on the plasma membrane. In eukaryotes, these oxidative processes are localized to the inner mitochondrial membrane, which is highly folded into cristae to increase surface area. This evolutionary shift reflects the endosymbiotic origin of mitochondria from an ancestral aerobic proteobacterium.

**21.** (B) II only
Eukaryotic cells contain 80S ribosomes in their cytosol, which are composed of 60S and 40S subunits. While eukaryotes do contain 70S ribosomes, these are located exclusively within their mitochondria and chloroplasts, reflecting their endosymbiotic history. In contrast, prokaryotes rely entirely on 70S ribosomes for protein synthesis. Therefore, the claim about cytosolic eukaryotic ribosomes is incorrect.

**22.** (C) Synthesis and assembly of ribosomal RNA and ribosomal subunits
The nucleolus is a dense, non-membrane-bound structure located within the eukaryotic nucleus. Its primary function is the transcription of ribosomal RNA genes and the subsequent assembly of these RNA molecules with proteins to form ribosomal subunits. These immature subunits are eventually exported through nuclear pores into the cytoplasm, where they combine to facilitate the translation of messenger RNA.

**23.** (D) Intermediate filaments
Intermediate filaments provide mechanical strength and maintain the shape of animal cells, which lack a rigid outer boundary. They form a stable network throughout the cytoplasm and anchor organelles. In plant cells, the rigid cell wall composed of cellulose provides the necessary structural support and protection, making the extensive intermediate filament networks found in animal cells less essential.

**24.** (A) Chitin
Fungal cell walls are chemically distinct from those of plants and bacteria. Their primary structural component is chitin, a tough nitrogen-containing polysaccharide also found in the exoskeletons of arthropods. This differs from plant cell walls, which are mainly cellulose, and bacterial cell walls, which contain peptidoglycan. Chitin provides the necessary rigidity and protection for fungal cells to survive.

**25.** (B) Singer and Nicolson
The fluid mosaic model, proposed by S.J. Singer and Garth Nicolson in 1972, describes the cell membrane as a dynamic, two-dimensional liquid. It suggests that the membrane is a mosaic of various proteins floating in or on a fluid lipid bilayer. This model revolutionized biological understanding by explaining how components move laterally while maintaining the integrity of the barrier.

**26.** (A) P-4, Q-2, R-1, S-3
Phospholipids form the basic bilayer matrix of the membrane, providing its fundamental structure. Cholesterol is interspersed within the tails to modulate fluidity. Integral proteins span the hydrophobic core to facilitate transport or signaling, while peripheral proteins remain bound to the membrane surface. This organization ensures the membrane can perform diverse biological functions while remaining stable and flexible.

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**27.** (A) The proportion of unsaturated fatty acid chains in phospholipids
Membrane fluidity is heavily influenced by the chemical structure of phospholipid tails. Unsaturated fatty acids contain double bonds that create "kinks," preventing the molecules from packing tightly together. This increases the space between lipids, allowing them to move more freely. Cells can adjust the ratio of saturated to unsaturated fats to maintain optimal fluidity under varying environmental temperatures.

**28.** (C) Microdomains rich in cholesterol, sphingolipids, and specific receptor proteins
Lipid rafts are specialized microdomains within the plasma membrane that are more ordered and less fluid than the surrounding bilayer. They are highly enriched in cholesterol and sphingolipids, which pack together tightly. These rafts serve as organizing centers for cell signaling and protein trafficking by concentrating specific receptors and signaling molecules, facilitating efficient communication between the cell and its environment.

**29.** (D) They possess hydrophobic domains that interact with the fatty acid tails of the bilayer.
Integral membrane proteins are permanently embedded within the lipid bilayer. To remain stable in this environment, they contain one or more hydrophobic domains, typically arranged as alpha-helices or beta-barrels. These nonpolar regions interact with the hydrophobic fatty acid tails of the phospholipids, while their hydrophilic portions extend into the aqueous environments of the cytosol or the extracellular space.

**30.** (C) Transverse diffusion (flip-flop) from one leaflet to the other
Phospholipid molecules move rapidly through lateral diffusion and rotation. However, moving from one leaflet of the bilayer to the opposite one is thermodynamically unfavorable because the hydrophilic head must pass through the hydrophobic core. This "flip-flop" movement occurs very rarely on its own and requires specialized enzymes like flippases and floppases to facilitate the process and maintain membrane asymmetry.

**31.** (B) I and II
Biological membranes exhibit a distinct asymmetry between their two leaflets. Glycolipids are found exclusively on the extracellular face, where they contribute to the glycocalyx. Meanwhile, phosphatidylserine is primarily kept in the cytosolic leaflet. This distribution is dynamic and can be purposefully scrambled during processes like apoptosis, where the appearance of phosphatidylserine on the outer surface signals phagocytosis.

**32.** (D) Peripheral membrane proteins
Peripheral proteins are not embedded within the hydrophobic core of the lipid bilayer. Instead, they are associated with the membrane through relatively weak electrostatic interactions or hydrogen bonding with integral proteins or phospholipid heads. Because they are not deeply integrated, they can be easily dissociated from the membrane using mild chemical treatments, such as high salt concentrations or pH adjustments.

**33.** (A) It facilitates cell-cell recognition, adhesion, and protection.
The glycocalyx is a carbohydrate-rich coating on the outer surface of animal cells, formed by the sugar chains of glycoproteins and glycolipids. This layer acts as a molecular "signature" that allows cells to recognize each other and interact. It also provides physical protection against mechanical damage and plays a vital role in immune system responses and cell adhesion.

**34.** (C) It restricts the movement of phospholipid fatty acid chains, decreasing fluidity.
At physiological temperatures, cholesterol acts as a bidirectional regulator of membrane fluidity. It wedges itself between the phospholipid tails, restricting their lateral movement and making the membrane less fluid and more stable. However, at lower temperatures, it prevents the tails from packing too tightly, preventing the membrane from freezing. Thus, it ensures the membrane remains functional across different conditions.

**35.** (A) They are situated exclusively on the cytosolic face of the plasma membrane.
Membrane carbohydrates are found exclusively on the extracellular face of the plasma membrane, never on the cytosolic side. They are covalently attached to proteins or lipids, forming glycoproteins and glycolipids. These sugars are essential for cell-cell communication, serving as recognition sites for hormones and antibodies, and they also contribute to the protective carbohydrate coat known as the glycocalyx.

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**36.** (D) Hydrophobic alpha-helices
Multipass integral membrane proteins typically cross the lipid bilayer using hydrophobic alpha-helices. The side chains of the amino acids in these helices are nonpolar, allowing them to interact favorably with the fatty acid tails of the phospholipids. This structural motif provides a stable way for the protein to span the membrane multiple times, often forming channels or receptor structures.

**37.** (B) Actin filaments, microtubules, and intermediate filaments
The eukaryotic cytoskeleton is a dynamic network of protein filaments that provides structural support and facilitates movement. It consists of three primary components: microfilaments (actin), which are involved in cell shape and motility; microtubules (tubulin), which organize the cytoplasm and move organelles; and intermediate filaments, which provide mechanical strength. Together, these elements allow the cell to maintain its integrity.

**38.** (C) Amoeboid movement and formation of pseudopodia
Microfilaments, composed of actin subunits, are highly dynamic and can rapidly assemble or disassemble. This flexibility allows them to drive cellular movements, such as the formation of pseudopodia in amoebae and macrophages. By polymerizing actin at the leading edge and contracting the rear using myosin, cells can crawl across surfaces and change their shape in response to environmental stimuli.

**39.** (D) Alpha and beta tubulin heterodimers
Microtubules are hollow tubes formed by the polymerization of globular tubulin proteins. The basic building block is a heterodimer consisting of one alpha-tubulin and one beta-tubulin subunit. These dimers align end-to-end to form protofilaments, and typically thirteen protofilaments associate side-by-side to create the characteristic cylindrical structure. This organization provides the rigidity needed to act as tracks for intracellular transport.

**40.** (A) It allows for the rapid remodeling of the cytoskeleton, essential for cell division.
Dynamic instability is the ability of microtubules to switch between phases of slow growth and rapid shrinkage. This behavior is crucial during the cell cycle, particularly in mitosis, where the cytoskeleton must be completely reorganized to form the spindle apparatus. This rapid remodeling ensures that chromosomes are accurately captured and segregated into daughter cells during the process of division.

**41.** (A) II, IV and V
Intermediate filaments are unique among cytoskeletal elements because they are non-polar and do not require ATP or GTP for their assembly. Instead, they form stable, rope-like structures that provide mechanical resilience. While they are essential for structural integrity, they do not participate in the movement of cilia or the transport of cargo, which are functions reserved for microtubules and microfilaments.

**42.** (B) Myosin
Muscle contraction is the result of the coordinated interaction between actin microfilaments and the motor protein myosin. Myosin heads bind to actin and use the energy from ATP hydrolysis to pull the filaments past one another, shortening the sarcomere. This "sliding filament" mechanism is the fundamental process behind all voluntary and involuntary muscle movements in the human body.

**43.** (A) Nuclear lamins
Nuclear lamins are a specific class of intermediate filaments that form a dense, fibrous meshwork called the nuclear lamina. Located just beneath the inner nuclear membrane, this structure provides essential mechanical support to the nuclear envelope and organizes chromatin. Mutations in the genes encoding these lamins can lead to severe genetic disorders, including premature aging syndromes known as progerias.

**44.** (C) Kinesins generally move towards the plus end, while dyneins move towards the minus end.
Motor proteins utilize the structural polarity of microtubules to transport cellular cargo. Most kinesins move toward the fast-growing "plus" end, which is typically oriented toward the cell periphery. In contrast, cytoplasmic dyneins move toward the "minus" end, located at the microtubule-organizing center near the nucleus. This directional transport ensures that organelles and vesicles reach their correct destinations.

**45.** (D) The centrosome
In animal cells, the primary microtubule-organizing center (MTOC) is the centrosome,

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which is located near the nucleus. It contains a pair of centrioles surrounded by a cloud of pericentriolar material rich in gamma-tubulin. This gamma-tubulin acts as a template for the nucleation of new microtubules, determining their orientation and ensuring the proper organization of the cell's internal transport network.

**46.** (C) ATP
The polymerization of actin subunits into microfilaments is an energy-dependent process. Globular actin (G-actin) monomers bind to ATP before they can be added to the growing filament. Once integrated, the ATP is eventually hydrolyzed to ADP, which reduces the affinity of the subunit for its neighbors and promotes disassembly at the opposite end. This cycle drives the dynamic turnover of filaments.

**47.** (D) Colchicine
Colchicine is a potent drug that binds specifically to tubulin dimers, preventing them from polymerizing into microtubules. By disrupting microtubule formation, it inhibits the assembly of the mitotic spindle during cell division. This arrests the cell cycle in metaphase, making it a valuable tool in cytogenetics for karyotyping and in medicine for treating conditions like gout by reducing inflammatory cell movement.

**48.** (C) Microtubules — 25 nm
The cytoskeleton is composed of three main filaments categorized by their thickness. Microfilaments are the thinnest at approximately 7 nm in diameter. Intermediate filaments have an intermediate size of about 10 nm. Microtubules are the largest components, forming hollow cylinders with a diameter of roughly 25 nm. Understanding these dimensions is essential for visualizing the structural hierarchy within the eukaryotic cytoplasm.

**49.** (A) Ribosomes
The rough endoplasmic reticulum (RER) derives its name from its characteristic appearance under an electron microscope. Its cytosolic surface is studded with numerous ribosomes, which are the sites of active protein synthesis. These bound ribosomes translate mRNAs encoding proteins destined for secretion, membrane insertion, or lysosomal targeting, distinguishing the RER functionally and structurally from the ribosome-free smooth endoplasmic reticulum.

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**50.** (C) Biosynthesis of membrane lipids and steroid hormones
The smooth endoplasmic reticulum (SER) lacks ribosomes and specializes in various metabolic processes. It is the primary site for the synthesis of phospholipids and cholesterol required for all cellular membranes. Additionally, in specialized cells like those in the adrenal glands and gonads, the SER contains the enzymes necessary for the production of steroid hormones from a cholesterol precursor.

**51.** (B) Rough ER → Golgi → Secretory vesicle → Plasma membrane
The secretory pathway describes the journey of proteins intended for export or membrane delivery. Synthesis begins on ribosomes of the rough ER, where proteins enter the lumen. They are transported via vesicles to the Golgi apparatus for processing and sorting. Finally, they are packaged into secretory vesicles that fuse with the plasma membrane, releasing their contents through exocytosis.

**52.** (A) It functions as a dynamic intracellular storage site for calcium ions.
The sarcoplasmic reticulum is a specialized form of smooth ER found in muscle cells. Its primary function is the regulation of calcium levels in the cytoplasm. It actively pumps calcium ions from the cytosol into its lumen for storage and releases them rapidly in response to nerve impulses, triggering the interaction between actin and myosin that leads to muscle contraction.

**53.** (A) Dolichol phosphate
N-linked glycosylation begins in the rough ER with the assembly of a core oligosaccharide. This sugar chain is first built on dolichol phosphate, a specialized lipid molecule embedded in the ER membrane. Once the precursor is complete, an enzyme complex transfers it from the dolichol anchor to a specific asparagine residue on the growing polypeptide chain as it enters the ER.

**54.** (D) All translation of mRNA in a eukaryotic cell occurs on the rough ER.
While the rough ER is the site for synthesizing secretory and membrane proteins, it is not the only location for translation. Many proteins, such as those destined for the cytosol, nucleus, mitochondria, or peroxisomes, are translated by "free" ribosomes floating in the cytoplasm.

Therefore, the claim that all translation occurs on the rough ER is a biological misconception.

**55.** (A) They are translocated back to the cytosol for degradation by the proteasome (ERAD pathway).
The cell maintains a strict quality control system in the ER. Proteins that fail to fold correctly are recognized by chaperones and targeted for Endoplasmic Reticulum-Associated Degradation (ERAD). In this process, the misfolded proteins are exported back through the membrane into the cytosol, where they are tagged with ubiquitin and destroyed by the proteasome to prevent toxic protein aggregation.

**56.** (D) BiP (Binding Immunoglobulin Protein)
BiP is a major molecular chaperone located within the lumen of the rough endoplasmic reticulum. It belongs to the Hsp70 family and plays a vital role in protein folding. BiP binds to exposed hydrophobic patches on nascent polypeptide chains, preventing them from aggregating and guiding them toward their correct three-dimensional conformation before they can proceed through the secretory pathway.

**57.** (D) Smooth ER
Leydig cells are specialized cells in the testes responsible for the production of testosterone, a steroid hormone. Because steroid synthesis requires numerous enzymes localized in the smooth endoplasmic reticulum, these cells possess an exceptionally well-developed SER network. This structural adaptation allows the cells to efficiently process cholesterol and other lipid precursors into the high volumes of hormones needed for male reproductive function.

**58.** (A) Co-translational translocation via the Sec61 channel
Secretory proteins are typically imported into the ER while they are still being synthesized by the ribosome. The ribosome docks onto the ER membrane at a specialized protein-conducting pore called the Sec61 translocon. As the polypeptide chain grows, it is threaded directly through this channel into the ER lumen, ensuring that the protein is properly sequestered from the cytosolic environment.

**59.** (B) Cytochrome P450
The smooth ER of liver hepatocytes is heavily involved in the detoxification of various lipid-soluble drugs and toxic metabolites. This process is mediated by the cytochrome P450 family of enzymes. These enzymes chemically modify harmful substances to make them more water-soluble, which facilitates their excretion from the body. Consequently, chronic drug use can lead to a compensatory proliferation of the SER.

**60.** (B) By budding off transitional transport vesicles
The endoplasmic reticulum acts as the source of membrane and protein components for the rest of the endomembrane system. Specialized regions known as ER exit sites produce small, membrane-bound transport vesicles. These vesicles carry newly synthesized lipids and proteins to the Golgi apparatus. This continuous flow of vesicular traffic is essential for the maintenance, growth, and functional organization of the Golgi cisternae.

**61.** (A) $3 \rightarrow 1 \rightarrow 2$
During translation, tRNA molecules move sequentially through three distinct sites on the ribosome. A new aminoacyl-tRNA first enters the A (aminoacyl) site. After peptide bond formation, the ribosome shifts, moving the tRNA carrying the growing chain to the P (peptidyl) site. Finally, the "spent" tRNA moves to the E (exit) site, where it is released back into the cytosol for recharging.

**62.** (D) 60S and 40S subunits
Eukaryotic 80S ribosomes are complex molecular machines composed of two distinct subunits. The large subunit is designated as 60S, while the small subunit is 40S. These values represent their sedimentation rates in an ultracentrifuge. Although their mathematical sum is 100, the combined 80S value reflects the specific shape and density of the functional complex formed when the two subunits associate during translation.

**63.** (A) 80%
Ribosomal RNA (rRNA) is the most abundant type of RNA in the cell, typically constituting about 80% of the total RNA content. This high percentage reflects the massive number of ribosomes required to meet the protein synthesis demands of the cell. Unlike messenger RNA, which is transient and exists in small amounts, rRNA is a stable structural and catalytic component of the ribosomes.

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**64.** (D) It catalyzes the formation of peptide bonds (peptidyl transferase activity).
The 28S rRNA, found in the large subunit of eukaryotic ribosomes, functions as a ribozyme. It provides the catalytic peptidyl transferase activity required to link amino acids together via peptide bonds. This discovery was revolutionary because it demonstrated that the core catalytic function of the ribosome is performed by RNA rather than proteins, supporting the evolutionary concept of an early RNA-based world.

**65.** (B) The nucleolus
The nucleolus is the specialized sub-nuclear compartment dedicated to ribosome production. Here, ribosomal RNA genes are transcribed into large precursors that are subsequently processed into smaller rRNA species. These RNAs then associate with ribosomal proteins imported from the cytoplasm to form the large and small ribosomal subunits. Once partially assembled, these subunits are exported through nuclear pores to complete their maturation in the cytoplasm.

**66.** (D) They are composed of a 50S large subunit and a 30S small subunit.
Prokaryotic ribosomes are smaller and less complex than their eukaryotic counterparts. They have a total sedimentation coefficient of 70S and are composed of a 50S large subunit and a 30S small subunit. While they perform the same fundamental task of protein synthesis using tRNA and mRNA, their distinct protein and rRNA composition makes them specific targets for many important medical antibiotics.

**67.** (A) 18S rRNA
The small (40S) subunit of the eukaryotic ribosome contains a single molecule of 18S ribosomal RNA. This rRNA molecule plays a critical structural role and is involved in the correct positioning of the messenger RNA during the initiation of translation. The sequence of the 18S rRNA is highly conserved, making it a standard tool for scientists studying evolutionary relationships between different eukaryotic species.

**68.** (C) The 5' cap (m7G)
Translation initiation in eukaryotes begins with the recognition of the 5' methylated guanosine cap of the mRNA. The small 40S ribosomal subunit, associated with various initiation factors, binds to this cap structure. It then "scans" along the 5' untranslated region until it encounters the first AUG start codon, where it pauses to allow the large 60S subunit to join and begin synthesis.

**69.** (B) Aminoglycosides (e.g., Streptomycin)
Aminoglycosides are a class of antibiotics that specifically target the 30S subunit of bacterial ribosomes. By binding to the 16S rRNA within this subunit, they interfere with the accurate recognition of codons by tRNA. This results in the production of defective, misfolded proteins or the premature termination of translation, which ultimately leads to the death of the susceptible bacterial cell.

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**70.** (D) A complex of multiple ribosomes simultaneously translating a single mRNA molecule
A polyribosome, or polysome, consists of an mRNA molecule with several ribosomes attached to it at various stages of translation. This arrangement allows the cell to produce multiple copies of a protein from a single mRNA transcript simultaneously, greatly increasing the efficiency of protein synthesis. Polysomes can be found either free-floating in the cytosol or attached to the rough endoplasmic reticulum membrane.

**71.** (A) RNA polymerase III
In eukaryotes, most ribosomal RNAs are transcribed as a single large precursor by RNA polymerase I in the nucleolus. However, the 5S rRNA is an exception; it is encoded by separate genes located outside the nucleolus and is transcribed by RNA polymerase III. After its synthesis, the 5S rRNA is transported into the nucleolus to be incorporated into the assembling large ribosomal subunit.

**72.** (B) Through a translocon channel into the ER lumen
If a nascent protein possesses an N-terminal ER signal sequence, the entire ribosome-mRNA complex is targeted to the rough endoplasmic reticulum. The growing polypeptide chain is then threaded through a proteinaceous channel called the translocon. This ensures that the protein enters the ER lumen or becomes embedded in the ER membrane, directing it into the secretory pathway for further processing and delivery.

**73.** (D) Dictyosomes
In many plant cells, the Golgi apparatus does not form a single large complex like those found in animal cells. Instead, it consists of numerous small, independent stacks of cisternae distributed throughout the cytoplasm. These individual stacks are called dictyosomes. They perform the same essential functions of modifying and packaging proteins and cell wall polysaccharides but operate as separate units within the plant's cytoplasmic matrix.

**74.** (C) The cis face
The Golgi apparatus exhibits structural polarity. The cis face, or entry face, is typically convex and oriented toward the endoplasmic reticulum. It receives transport vesicles containing newly synthesized proteins and lipids from the ER. Upon fusion, these molecules enter the Golgi cisternae to undergo a series of sequential post-translational modifications as they move through the medial region toward the opposite trans face.

**75.** (C) Complex O-linked glycosylation
The Golgi apparatus is the primary site for O-linked glycosylation, where sugar molecules are attached to the oxygen atom of serine or threonine residues. This process is distinct from N-linked glycosylation, which begins in the ER. The Golgi enzymes add specific sugars in a defined order to create complex carbohydrate structures that are essential for protein stability, recognition, and various extracellular functions.

**76.** (A) Both A and R are true and R is the correct explanation of A.
Lysosomal enzymes are specifically modified in the cis-Golgi through the addition of a mannose-6-phosphate (M6P) tag. This chemical marker acts as a sorting signal. When these enzymes reach the trans-Golgi network, they are recognized by M6P receptors, which package them into clathrin-coated vesicles destined for the endosomal-lysosomal system. This precise mechanism ensures that digestive enzymes do not reach the wrong location.

**77.** (C) COPI-coated vesicles
Retrograde transport refers to the movement of vesicles "backward" through the secretory pathway. COPI-coated vesicles are responsible for this process, carrying escaped ER-resident proteins and recycled membrane components from the Golgi back to the endoplasmic reticulum. This retrieval mechanism is essential for maintaining the distinct protein compositions and functional identities of both the ER and the various Golgi compartments within the cell.

**78.** (C) Proteins are sorted and packaged into specific vesicles destined for different cellular locations.
The trans-Golgi network (TGN) is the final sorting hub of the Golgi apparatus. Here, fully processed proteins and lipids are segregated into different transport vesicles based on their molecular signals. These vesicles are then directed to specific destinations, including the plasma membrane for secretion, the extracellular matrix, or the lysosome for degradation, ensuring the cell correctly distributes its synthesized products.

**79.** (A) It synthesizes the core oligosaccharide tree linked to asparagine.
The synthesis of the core oligosaccharide tree for N-linked glycosylation is a process that occurs exclusively in the endoplasmic reticulum, not the Golgi. The Golgi's role in glycosylation involves the subsequent trimming and addition of different sugar residues to this pre-existing core to create complex glycans. Thus, the statement claiming the Golgi synthesizes the initial core oligosaccharide is biologically incorrect.

**80.** (C) Vesicular transport model
The vesicular transport model proposes that the Golgi cisternae are stable, long-lived structures. According to this view, proteins and lipids move forward through the Golgi stack via small transport vesicles that bud from one cisterna and fuse with the next. This model contrasts with the cisternal maturation model, where the cisternae themselves physically move and transform from cis to trans over time.

**81.** (B) Glycosyltransferases
Glycosyltransferases are a diverse family of enzymes localized within the Golgi cisternae. They catalyze the transfer of sugar moieties from activated nucleotide-sugar donors to specific acceptor molecules, such as growing carbohydrate chains on proteins or lipids. The sequential action of different glycosyltransferases, each specific for a particular sugar and linkage, allows the Golgi to generate a vast array of complex oligosaccharide structures.

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**82.** (D) Golgi apparatus
While the basic phospholipid matrix is synthesized in the ER, the Golgi apparatus is the site for the production of specialized lipids. Ceramide, synthesized in the ER, is transported to the Golgi, where it is converted into sphingomyelin and various glycolipids. These molecules are essential components of the plasma membrane, particularly in forming lipid rafts that are involved in signaling and transport.

**83.** (A) Secretory (exocytic) pathway
The secretory pathway represents the fundamental flow of membrane and proteins from their site of synthesis to the cell surface. This anterograde transport starts at the ER, moves through the Golgi for processing, and ends at the plasma membrane. It facilitates the delivery of new membrane lipids and the release of hormones, enzymes, and other cargo into the extracellular environment via exocytosis.

**84.** (D) Secretory vesicles or constitutive transport vesicles
Proteins destined for the plasma membrane are packaged at the trans-Golgi network into specific vesicles. These can be constitutive transport vesicles, which provide a continuous supply of membrane components, or regulated secretory vesicles, which store concentrated cargo until a signal triggers their release. These vesicles move along the cytoskeleton and fuse with the cell surface, completing the final step of the secretory pathway.

**85.** (A) Cristae
The inner mitochondrial membrane is folded into numerous shelF-like or tubular invaginations called cristae. This structural adaptation provides a massive surface area for the housing of electron transport chain complexes and ATP synthase enzymes. By maximizing this area, the mitochondrion can significantly increase its capacity for aerobic respiration and the production of ATP, meeting the high energy demands of eukaryotic cells.

**86.** (A) P-3, Q-4, R-1, S-2
Mitochondria have four distinct regions with specific functions. The outer membrane contains porins for permeability. The inner membrane houses the electron transport chain. The intermembrane space accumulates protons to create an electrochemical gradient. Finally, the matrix is the site of the Krebs cycle and contains the organelle's DNA. This compartmentalization is essential for the efficient conversion of chemical energy into ATP.

**87.** (A) Presence of large pore-forming proteins called porins
The outer mitochondrial membrane is remarkably permeable to small molecules and ions because it contains large amounts of porin proteins. These porins form aqueous channels that allow the free passage of molecules up to 5000 Daltons. In contrast, the inner membrane is highly impermeable and lacks porins, requiring specific transport proteins to move substances, which is critical for maintaining the proton gradient.

**88.** (D) Matrix
The mitochondrial matrix is the fluid-filled central space of the organelle. It contains a high concentration of specialized enzymes required for the citric acid cycle (Krebs cycle) and the beta-oxidation of fatty acids. These pathways break down metabolic fuels to produce high-energy electron carriers, such as NADH and $FADH_2$, which then deliver electrons to the respiratory chain located on the adjacent inner membrane.

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**89.** (A) Cardiolipin
Cardiolipin is a unique "double" phospholipid found almost exclusively in the inner mitochondrial membrane. It represents about 20% of the total lipid composition in this region. Cardiolipin is essential for the structural integrity and optimal activity of the respiratory complexes and ATP synthase. It also helps to minimize proton leakage across the membrane, thereby maintaining the electrochemical gradient necessary for efficient ATP synthesis.

**90.** (D) The intermembrane space
As electrons flow through the electron transport chain on the inner mitochondrial membrane, the energy released is used to pump protons ($H^+$) from the matrix into the narrow intermembrane space. This creates a high concentration of protons in the space, establishing a strong electrochemical gradient. This stored potential energy, known as the proton-motive force, eventually drives the synthesis of ATP through the process of chemiosmosis.

**91.** (D) It follows strictly Mendelian inheritance in most mammals.
Mitochondrial DNA (mtDNA) does not follow Mendelian inheritance patterns. In most mammals, including humans, mitochondria are inherited almost exclusively from the mother because the sperm's mitochondria are typically destroyed after fertilization. This uniparental inheritance means that mitochondrial genetic traits and diseases are passed down through the maternal line, distinguishing mtDNA fundamentally from the biparental inheritance of the nuclear genome.

**92.** (C) Matrix
ATP synthase is a complex molecular motor embedded in the inner mitochondrial membrane. It consists of two main parts: the F0 base within the membrane and the globular $F_1$ head. The $F_1$ portion, which contains the catalytic sites that synthesize ATP from ADP and inorganic phosphate, projects directly into the mitochondrial matrix. This allows newly synthesized ATP to be immediately available for various metabolic processes.

**93.** (C) Molecular oxygen ($O_2$)
In aerobic respiration, molecular oxygen acts as the final electron acceptor at the end of the electron transport chain. In the final step, cytochrome c oxidase (Complex IV) transfers electrons to oxygen, which then combines with protons from the matrix to form water. This continuous removal of electrons by oxygen is essential to keep the entire chain functioning and maintaining the proton gradient for ATP production.

**94.** (D) Apoptosis
Beyond energy production, mitochondria are central to regulated cell death, or apoptosis. In response to severe cellular stress, the outer mitochondrial membrane becomes permeable, allowing cytochrome c to leak from the intermembrane space into the cytosol. Once in the cytoplasm, cytochrome c binds to adaptor proteins to form the apoptosome, which activates a cascade of caspases that systematically dismantle the cell.

**95.** (A) Growth and binary fission of pre-existing mitochondria
New mitochondria are not created from scratch; instead, they are produced through the growth and division of pre-existing ones. This process, known as mitochondrial fission, is similar to bacterial binary fission and is coordinated with the cell's energy needs and the cell cycle. This semi-autonomous behavior reflects the evolutionary history of mitochondria as formerly independent prokaryotic organisms that became integrated into eukaryotic cells.

**96.** (D) Complex II (Succinate dehydrogenase)
Complex II, also known as succinate dehydrogenase, is an integral part of both the citric acid cycle and the electron transport chain. While it transfers electrons from succinate to the ubiquinone pool, it does not release enough energy during this process to pump protons across the inner mitochondrial membrane. Consequently, Complex II does not contribute directly to the establishment of the proton gradient used for ATP synthesis.

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**97.** (A) An internal system of interconnected membranous sacs called thylakoids
While both mitochondria and chloroplasts have double membranes and their own DNA, chloroplasts possess a unique third membrane system. This system consists of flattened, sac-like structures called thylakoids, which are often stacked into grana. The thylakoid membranes contain chlorophyll and the photosynthetic machinery required for the light-dependent reactions. This complex internal architecture is a defining feature of chloroplasts and is entirely absent in mitochondria.

**98.** (D) Amyloplasts
Amyloplasts are a specialized type of leucoplast, a category of non-pigmented plastids. They are primarily found in starch-storing tissues such as potato tubers, seeds, and roots. Amyloplasts synthesize and store starch granules (amylopectin and amylose) from glucose produced during photosynthesis. In some tissues, such as the root cap, amyloplasts also act as statoliths to help the plant sense gravity and guide root growth.

**99.** (A) Tonoplast
The tonoplast is the specialized semi-permeable membrane that encloses the large central vacuole in plant cells. It regulates the movement of ions, water, and various metabolites into and out of the vacuole. By actively transporting solutes, the tonoplast helps maintain the vacuole's osmotic pressure, which is essential for generating the

turgor pressure that provides structural support and rigidity to the entire plant.

**100.** (D) Degradation of long-chain fatty acids via beta-oxidation
Peroxisomes are multifunctional organelles involved in various lipid metabolic pathways. One of their most critical roles is the initial breakdown of very-long-chain fatty acids through the process of beta-oxidation. This pathway shortens the fatty acids before they are sent to the mitochondria for complete oxidation. In the process, peroxisomes generate hydrogen peroxide, which is then safely neutralized by the resident enzyme catalase.

**101.** (A) I and III
Peroxisomes and glyoxysomes are types of microbodies found in eukaryotic cells. Peroxisomes use catalase to break down hydrogen peroxide, protecting the cell from oxidative damage. Glyoxysomes are specialized versions found in plant seeds, where they harbor the enzymes of the glyoxylate cycle. This pathway is essential for converting stored lipids into sugars, providing the energy and carbon skeletons needed for the developing seedling to grow.

**102.** (C) The thylakoid membrane
The light-dependent reactions of photosynthesis occur exclusively within the thylakoid membranes of the chloroplast. These membranes contain the photosystems, electron transport chains, and ATP synthase complexes required to capture solar energy. During this process, light energy is converted into chemical energy in the form of ATP and NADPH, while water is split to release oxygen as a byproduct for the atmosphere.

**103.** (A) Stroma
RuBisCO is the most abundant enzyme on Earth and is central to the process of carbon fixation. It is located in the stroma, the fluid-filled space surrounding the thylakoid stacks within the chloroplast. In this region, RuBisCO catalyzes the first step of the Calvin cycle, where carbon dioxide is attached to a five-carbon sugar, eventually leading to the synthesis of glucose and other carbohydrates.

**104.** (D) The ability to synthesize glucose from acetyl-CoA derived from fat stores
The glyoxylate cycle, occurring in the glyoxysomes of germinating oilseeds, allows plants to bypass the carbon-releasing steps of the Krebs cycle. This enables the conversion of two-carbon acetyl-CoA molecules, derived from the breakdown of stored lipids, into four-carbon succinate. The succinate is then used to synthesize glucose via gluconeogenesis, providing the seedling with essential nutrients before it can begin performing photosynthesis.

**105.** (B) Solutes and ions
To maintain turgor pressure, plant cells must keep the central vacuole hypertonic compared to the surrounding cytoplasm. This is achieved by the active transport of various solutes, such as potassium ions, sugars, and organic acids, across the tonoplast. The resulting high solute concentration draws water into the vacuole via osmosis, creating the internal pressure necessary to keep the plant cell firm and upright.

**106.** (D) Plastid interconversions strictly require genetic material transfer from the nucleus.
Plastids are remarkably versatile and can transform from one type to another in response to environmental cues or developmental signals. For example, chloroplasts can become chromoplasts during fruit ripening, or amyloplasts can turn green when exposed to light. These interconversions involve changes in internal membrane structure and enzyme composition but do not require the transfer of new genetic material from the cell nucleus.

**107.** (A) The peroxisome
Photorespiration is a complex metabolic process that occurs when RuBisCO binds to oxygen instead of carbon dioxide. To recover the carbon lost during this wasteful reaction, the plant must coordinate a multi-organelle pathway involving the chloroplast, the peroxisome, and the mitochondrion. In the peroxisome, specific enzymes convert glycolate into glycine, which is then further processed in the mitochondria to minimize the loss of organic carbon.

**108.** (C) Stroma
The stroma is the dense, protein-rich fluid that fills the inner compartment of the chloroplast, surrounding the thylakoid network. It is functionally analogous to the mitochondrial matrix. The stroma contains the chloroplast's genome, ribosomes, and all the enzymes necessary for the Calvin cycle. It is the site where the

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energy-rich products of the light reactions (ATP and NADPH) are used to fix carbon.

**109.** (A) Acidic (pH ~4.5 to 5.0)
Lysosomes are the primary digestive organelles of the cell and contain a suite of roughly fifty different enzymes. These enzymes, known as acid hydrolases, have evolved to function optimally in a highly acidic environment, typically around pH 5.0. This requirement serves as a safety mechanism; if a lysosome were to rupture, the enzymes would become inactive in the neutral pH of the cytosol.

**110.** (B) Action of an ATP-dependent vacuolar-type $H^+$ pump (V-ATPase)
To maintain its internal acidity, the lysosomal membrane contains specialized protein complexes called V-ATPases. These pumps utilize the energy released from ATP hydrolysis to actively transport hydrogen ions (protons) from the cytosol into the lysosome against their concentration gradient. This continuous pumping ensures the internal pH remains low, providing the ideal conditions for the acid hydrolases to break down various macromolecules and debris.

**111.** (A) I and IV
Lysosomes contain acid hydrolases, not oxidases, to perform their digestive functions. Furthermore, a primary lysosome is characterized as a newly formed vesicle that contains inactive enzymes and has not yet engaged in digestion. Once it fuses with an endosome or phagosome to begin breaking down material, it becomes a secondary lysosome. Therefore, both the first and fourth statements provided in the question are incorrect.

**112.** (A) Acid hydrolases
The enzymes within lysosomes are collectively known as acid hydrolases because they use water to break chemical bonds and require an acidic environment for activity. This group includes proteases, nucleases, glycosidases, lipases, and phosphatases. Together, they are capable of degrading almost every type of biological polymer, including proteins, nucleic acids, carbohydrates, and lipids, into their fundamental building blocks for recycling within the cell.

**113.** (C) Autophagy
Autophagy is a highly regulated "selF-eating" process used by the cell to maintain homeostasis. During this process, damaged organelles, misfolded proteins, or excess cytoplasm are sequestered within a double-membrane vesicle. This vesicle eventually fuses with a lysosome, where its contents are degraded by acid hydrolases. Autophagy is essential for cell survival during nutrient deprivation and for the removal of potentially toxic cellular components.

**114.** (A) Autophagosome
Macroautophagy begins with the formation of a flat, cup-shaped membrane called a phagophore. This membrane expands and eventually closes to completely surround the target material, forming a double-membrane-bound vesicle known as an autophagosome. Once complete, the autophagosome moves along the cytoskeleton to fuse with a lysosome, delivering its cargo to the digestive enzymes for final breakdown and recycling of the constituent molecules.

**115.** (D) They remain in vesicles called residual bodies, which may undergo exocytosis or accumulate as lipofuscin.
After the digestion of cellular or foreign material is complete, any indigestible substances remain within the lysosome. This mature structure is referred to as a residual body. In some cells, these bodies can expel their contents through exocytosis. In others, especially long-lived cells like neurons, the material accumulates as a yellowish-brown pigment called lipofuscin, which is considered a hallmark of cellular aging.

**116.** (D) Lysosomal storage disorders (e.g., Tay-Sachs disease)
Lysosomal storage disorders are a group of genetic diseases caused by the malfunction of specific lysosomal enzymes. When an enzyme is missing or defective, its substrate cannot be broken down and instead accumulates within the lysosomes, eventually interfering with normal cell function. Examples include Tay-Sachs disease, Gaucher disease, and Pompe disease. These conditions often lead to severe organ dysfunction, skeletal abnormalities, and neurological decline.

**117.** (A) The internal membrane proteins are highly glycosylated, providing a protective carbohydrate shield.
The lysosome must protect its own membrane from being destroyed by its powerful acid hydrolases. This is achieved through a specialized glycocalyx-like coating on the inner surface of the lysosomal membrane. The integral membrane

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proteins are heavily modified with complex carbohydrate chains that face the lumen. These sugars form a physical barrier that prevents the digestive enzymes from reaching and degrading the underlying phospholipid bilayer.

**118.** (D) A newly budded vesicle from the Golgi containing inactive or pre-active digestive enzymes
A primary lysosome is the initial stage of the organelle's life cycle. It is a small, membrane-bound vesicle that has budded off from the trans-Golgi network. At this stage, it contains a full complement of acid hydrolases but has not yet encountered any substrate for digestion. It remains in this "resting" state until it fuses with an endocytic or autophagic vesicle to become a secondary lysosome.

**119.** (A) A mannose-6-phosphate (M6P) tag added in the Golgi
The targeting of enzymes to the lysosome depends on a specific carbohydrate signal. In the cis-Golgi, lysosomal proteins are recognized by their three-dimensional structure and modified by the addition of mannose-6-phosphate tags. These tags are then recognized by M6P receptors in the trans-Golgi network, which bundle the enzymes into specific transport vesicles. This sorting mechanism ensures that these potentially dangerous enzymes are not accidentally secreted.

**120.** (D) Autophagy
During periods of starvation or nutrient stress, the cell activates autophagy as a survival strategy. By breaking down non-essential proteins and surplus organelles, the cell can generate a steady supply of amino acids, fatty acids, and simple sugars. These recycled components are used to maintain vital metabolic processes and synthesize essential proteins, allowing the cell to endure harsh conditions until external nutrient sources become available once again.

**121.** (A) I, II and V
The nucleus is defined by its double-membrane nuclear envelope. The outer membrane is physically continuous with the rough endoplasmic reticulum, while nuclear pore complexes serve as the regulated gateways for molecular transport. However, the nuclear lamina is located on the inner face of the envelope, and the nucleolus is a non-membrane-bound sub-compartment. Therefore, only statements I, II, and V are biologically accurate.

**122.** (D) Nuclear lamina
The nuclear lamina is a dense protein meshwork that lines the inner surface of the nuclear envelope. Composed primarily of intermediate filaments called lamins, it provides essential mechanical support to the nucleus, maintaining its shape and protecting the genomic DNA. Beyond its structural role, the lamina serves as an attachment site for chromatin and participates in various nuclear processes, including DNA replication and gene regulation.

**123.** (D) Nuclear pore complexes (NPCs)
Nuclear pore complexes are massive, multi-protein channels that span both layers of the nuclear envelope. They act as the sole checkpoints for the exchange of macromolecules between the nucleus and the cytoplasm. While small molecules can diffuse through, large structures like mRNA transcripts and ribosomal subunits require specific transport receptors to pass through the NPC. This regulated transport is fundamental for the separation of transcription and translation.

**124.** (A) Assembly and modification of small nuclear ribonucleoproteins (snRNPs)
Cajal bodies are small, spherical sub-nuclear organelles found in the nucleoplasm of metabolically active cells. They are not bound by a membrane but are organized around specific protein and RNA components. Their primary function is the maturation and assembly of snRNPs, which are essential components of the spliceosome. By concentrating these molecules, Cajal bodies facilitate the efficient processing of pre-messenger RNA within the eukaryotic nucleus.

**125.** (C) It is enclosed by a selectively permeable phospholipid bilayer.
The nucleolus is a prominent example of a biomolecular condensate; it is not surrounded by a phospholipid membrane. Instead, it is formed through phase separation of proteins and RNA within the nucleoplasm. Its structure is maintained by the active transcription of ribosomal RNA and the assembly of ribosomal subunits. The lack of a membrane allows for the rapid exchange of components with the surrounding nuclear environment.

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**126.** (D) Histones
Histones are small, highly basic proteins that serve as the primary structural organizers of eukaryotic DNA. They form octameric complexes around which DNA wraps to create nucleosomes, the "beads-on-a-string" structure of chromatin. This packaging is essential for condensing the massive length of the genome into the small volume of the nucleus. Additionally, chemical modifications to histones play a central role in regulating gene accessibility and expression.

**127.** (A) Less condensed and actively undergoing transcription
Euchromatin represents the more open and relaxed form of chromatin within the nucleus. Because the DNA is less tightly packed around histones, it is more accessible to RNA polymerase and transcription factors. Consequently, euchromatin contains the majority of the cell's active genes. Its state can be dynamically regulated, allowing the cell to turn genes on or off in response to various internal and external signals.

**128.** (C) It serves as a structural scaffold organizing chromatin and aiding in transcription and replication.
The nuclear matrix is the insoluble proteinaceous framework that remains in the nucleus after the removal of DNA and histones. It acts as a three-dimensional scaffold that organizes chromatin into functional loops and provides docking sites for the molecular machinery involved in DNA replication and RNA synthesis. By providing this spatial organization, the nuclear matrix ensures that complex genetic processes are carried out in an orderly fashion.

**129.** (D) Passive diffusion
The nuclear pore complex contains an aqueous central channel that allows for the non-selective passage of small molecules. Ions, water, and small proteins with a molecular weight below approximately 40 kDa can move freely through these pores via passive diffusion. This allows the nucleus to maintain osmotic balance with the cytoplasm. In contrast, larger macromolecules must be actively transported using energy and specific transport receptors.

**130.** (D) Progeria and various muscular dystrophies
Mutations in the genes encoding nuclear lamins or the proteins that interact with them lead to a diverse group of disorders known as laminopathies. One of the most famous examples is Hutchinson-Gilford Progeria Syndrome, which causes accelerated aging in children. Other laminopathies include various forms of muscular dystrophy and lipodystrophy. these diseases highlight the critical importance of the nuclear lamina in maintaining genomic stability and cellular health.

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**131.** (C) Telophase
During telophase, the final stage of mitosis, the cell begins to rebuild its two new nuclei. Transport vesicles derived from the old nuclear envelope associate with the surface of the individual chromosomes, which have already reached the poles. These vesicles fuse to form a continuous double membrane, and nuclear pore complexes are reinserted. This process effectively sequesters the newly segregated genetic material from the cytoplasm before cytokinesis.

**132.** (D) Pre-ribosomal particles undergo assembly and maturation.
The granular component is the outermost region of the nucleolus. It contains a high concentration of maturing ribosomal subunits at various stages of assembly. In this area, newly synthesized ribosomal RNA, which has been processed in the fibrillar regions, associates with numerous ribosomal proteins. Once these large and small subunits are fully assembled and have reached the correct level of maturity, they are exported to the cytoplasm.

**133.** (A) Primary active transport
Primary active transport is a cellular process that moves ions or molecules across a membrane against their concentration gradient. This mechanism requires a direct input of metabolic energy, which is almost always provided by the hydrolysis of ATP. The transporter protein, often called a "pump," undergoes a conformational change that allows it to physically move the substrate from an area of low concentration to high concentration.

**134.** (C) Small charged ions (e.g., $Na^+$, $Cl^-$)
The hydrophobic core of the lipid bilayer acts as a highly effective barrier against charged particles. Despite their small size, ions like sodium, potassium, and chloride cannot pass through the nonpolar fatty acid tails of the membrane via simple diffusion. Their charge

creates a high energy barrier for entry into the hydrophobic environment, necessitating the use of specialized channel or carrier proteins for their transport.

**135.** (A) It utilizes specific integral membrane proteins (channels or carriers) to cross the membrane.
Facilitated diffusion is a form of passive transport that moves substances down their concentration gradient without the use of metabolic energy. Unlike simple diffusion, it requires the assistance of specific transmembrane proteins, such as ion channels or carrier proteins like glucose transporters. This allows the cell to move large or polar molecules that cannot otherwise penetrate the hydrophobic core of the lipid bilayer at a rapid rate.

**136.** (A) Both A and R are true and R is the correct explanation of A.
The sodium-potassium pump is a vital primary active transporter in animal cells. By using ATP to export three sodium ions for every two potassium ions imported, it creates an unequal distribution of charge across the membrane. This process makes the cell interior more negative and maintains the steep concentration gradients necessary for generating nerve impulses and regulating cell volume, thus directly establishing the resting membrane potential.

**137.** (A) They are specialized channel proteins that rapidly facilitate the diffusion of water across the membrane.
Aquaporins are integral membrane proteins that form highly selective pores specifically for water molecules. While water can slowly diffuse through the lipid bilayer, aquaporins allow for much more rapid transport, which is essential in tissues like the kidney and red blood cells. These channels exclude ions and other solutes, ensuring that only pure water moves across the membrane in response to existing osmotic gradients.

**138.** (D) They utilize ATP-binding cassettes to actively transport various substrates across membranes.
ATP-Binding Cassette (ABC) transporters are a superfamily of proteins that use the energy from ATP hydrolysis to move a wide variety of molecules across membranes. They possess a characteristic pair of highly conserved ATP-binding domains. These transporters can move everything from small ions and sugars to large lipids and drugs, and they play significant roles in both normal cellular physiology and clinical drug resistance.

**139.** (A) Secondary active transport (symport)
Secondary active transport does not use ATP directly; instead, it utilizes the energy stored in an existing electrochemical gradient, usually created by a primary active transporter. In the case of glucose uptake in the intestine, the transporter moves glucose and sodium into the cell together. The "downhill" movement of sodium provides the energy to pull glucose "uphill" against its own concentration gradient in a symport mechanism.

**140.** (D) Lower solute concentration to higher solute concentration
Osmosis is a specific type of passive transport involving the movement of water across a semi-permeable membrane. Water molecules move from a region of low solute concentration (high water potential) to a region of high solute concentration (low water potential). This process continues until the solute concentrations on both sides of the membrane are equalized or until external pressure counteracts the osmotic movement.

**141.** (D) They transport ions at rates much slower than carrier proteins.
Ion channels are highly efficient transporters that can move millions of ions per second. When an ion channel opens, it forms a continuous aqueous pore that allows ions to flow rapidly down their electrochemical gradient. This rate is significantly faster than that of carrier proteins, which must undergo a time-consuming conformational change for every molecule they transport. Therefore, the statement about slower transport rates is incorrect.

**142.** (A) The cell will lose water and shrink (crenation).
When a red blood cell is placed in a hypertonic solution, the surrounding fluid has a higher concentration of solutes than the cell's cytoplasm. To reach equilibrium, water moves out of the cell via osmosis. This loss of internal fluid causes the cell to shrivel and develop a scalloped or "crenated" appearance. If the water loss is severe, the cell may lose its functional capacity and die.

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**143.** (D) The $Na^+/Ca^{2+}$ exchanger, which moves $Ca^{2+}$ out of the cell while allowing $Na^+$ to enter
Antiport is a type of secondary active transport where two different substances are moved across the membrane in opposite directions. The sodium-calcium exchanger is a classic example; it uses the inward flow of sodium ions down their concentration gradient to drive the outward transport of calcium ions. This mechanism is crucial for maintaining low intracellular calcium levels, particularly in cardiac muscle cells, after a contraction event.

**144.** (C) ABC transporters
The multidrug resistance (MDR) protein, also known as P-glycoprotein, is a member of the ABC transporter family. It uses ATP energy to pump diverse hydrophobic drugs and toxins out of the cell. In cancer cells, high expression of this protein can lead to the failure of chemotherapy, as the drugs are expelled before they can reach their targets and kill the tumor cells, representing a major clinical challenge.

**145.** (C) Exocytosis
Exocytosis is the process of bulk transport used by cells to release substances into the extracellular space. Cargo molecules, such as hormones or neurotransmitters, are packaged into membrane-bound secretory vesicles. When these vesicles reach the plasma membrane, they fuse with it, opening to the outside and discharging their contents. This process is also essential for adding new lipids and proteins to the cell's outer membrane.

**146.** (A) P-3, Q-2, R-4, S-1
Bulk transport mechanisms are categorized by their function and specificity. Phagocytosis involves engulfing large particles. Pinocytosis is the non-specific uptake of extracellular fluid. Receptor-mediated endocytosis is highly specific, using clathrin to internalize particular ligands. Finally, exocytosis is the release of substances, such as neurotransmitters, from the cell. These processes allow the cell to interact with and respond to its environment on a large scale.

**147.** (A) Clathrin
Clathrin is a protein that plays a central role in the formation of coated vesicles for receptor-mediated endocytosis. It forms a unique "triskelion" structure. As these triskelions assemble on the inner face of the plasma membrane, they create a lattice that forces the membrane to curve inward, forming a pit. This lattice provides the structural framework necessary to package specific receptor-ligand complexes into a new vesicle.

**148.** (C) Dynamin
Dynamin is a specialized GTPase enzyme essential for the final step of vesicle formation during endocytosis. As the clathrin-coated pit invaginates, dynamin molecules polymerize around the neck of the budding vesicle. Hydrolysis of GTP provides the energy needed for dynamin to constrict and pinch off the vesicle from the plasma membrane, releasing it into the cytosol. Without dynamin, vesicles remain attached to the membrane and cannot internalize.

**149.** (A) Phagocytosis
Phagocytosis, or "cell eating," is a vital immune defense mechanism utilized by specialized cells like macrophages and neutrophils. These cells extend large cytoplasmic projections called pseudopodia to surround and engulf invading bacteria or large cellular debris. The resulting internal vesicle, known as a phagosome, then fuses with a lysosome, where the trapped pathogen is destroyed by digestive enzymes and toxic oxygen radicals.

**150.** (D) Constitutive exocytosis operates continuously without an external signal, whereas regulated exocytosis requires a specific trigger.
Cells utilize two main pathways for exocytosis. Constitutive exocytosis is a continuous process used to supply the plasma membrane with new lipids and proteins and to secrete substances into the extracellular matrix. Regulated exocytosis, however, only occurs in specialized cells. In this pathway, secretory products are stored in vesicles and are only released in response to a specific external signal, such as a hormone or nerve impulse.

**151.** (A) Ligand binding → Clathrin pit formation → Dynamin cleavage → Uncoating → Fusion with endosome
Receptor-mediated endocytosis follows a precise sequence. It begins with the ligand binding to its receptor, which then clusters in a clathrin-coated pit. The pit invaginates and is pinched off by dynamin to form a vesicle. Once inside the cell, the clathrin coat is removed (uncoating), and the vesicle fuses with an early endosome. This

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allows the cell to internalize specific molecules while recycling its receptors and membrane components.

**152.** (D) They mediate the specific docking and fusion of vesicles with their target membranes.
SNARE proteins are essential for ensuring that transport vesicles fuse with the correct target membrane. V-SNAREs located on the vesicle surface interact specifically with T-SNAREs on the target membrane. When these proteins match, they wrap around each other to form a tight bundle, pulling the two membranes close enough to overcome the repulsive forces and trigger fusion. This molecular "lock-and-key" system maintains the organization of the endomembrane system.

**153.** (A) It involves the continuous, non-specific uptake of small droplets of extracellular fluid.
Pinocytosis, or "cell drinking," is a form of endocytosis where the cell continuously samples the surrounding liquid environment. Small portions of the plasma membrane invaginate to form tiny vesicles, bringing in extracellular fluid and any dissolved solutes. Unlike phagocytosis or receptor-mediated endocytosis, pinocytosis is a non-selective process that occurs in almost all eukaryotic cells, helping to maintain membrane balance and provide a steady supply of nutrients.

**154.** (B) Receptor-mediated endocytosis of low-density lipoproteins (LDL)
Familial hypercholesterolemia is a genetic disorder characterized by dangerously high levels of cholesterol in the blood. It is caused by mutations in the LDL receptor or the proteins involved in clathrin-mediated endocytosis. Because the cells cannot efficiently internalize LDL particles through receptor-mediated endocytosis, cholesterol remains in the bloodstream, leading to premature atherosclerosis and an increased risk of heart attacks.

**155.** (A) Endocytosis on one side of a polarized cell followed by exocytosis on the opposite side
Transcytosis is a specialized transport mechanism used to move macromolecules across entire cellular barriers, such as the intestinal lining or the blood-brain barrier. It involves internalizing a substance through endocytosis on one side of a polarized cell, transporting the vesicle across the cytoplasm, and then releasing the contents through exocytosis on the opposite side. This process allows the body to move large molecules like antibodies between different compartments.

**156.** (D) Regulated exocytosis
The release of insulin from the pancreas is a classic example of regulated exocytosis. Pancreatic beta cells synthesize and package insulin into specialized secretory vesicles. These vesicles remain stored in the cytoplasm until blood glucose levels rise. The influx of glucose triggers a signaling cascade that causes the vesicles to fuse with the plasma membrane, releasing a burst of insulin into the bloodstream to maintain glucose homeostasis.

**157.** (A) Signal Recognition Particle (SRP)
The Signal Recognition Particle (SRP) is a ribonucleoprotein complex that acts as a molecular "postal worker." It specifically recognizes the hydrophobic signal sequence at the N-terminus of proteins destined for the secretory pathway as they emerge from the ribosome. Upon binding, SRP guides the entire ribosome-mRNA complex to the endoplasmic reticulum, ensuring that the protein is correctly targeted for translocation into the ER lumen.

**158.** (D) It temporarily pauses translation until the ribosome docks at the ER membrane.
When the Signal Recognition Particle (SRP) binds to the signal peptide and the ribosome, it induces a conformational change that halts further elongation of the polypeptide chain. This "translational arrest" is a critical safety mechanism. It prevents the protein from being released into the cytosol, where it might misfold or cause damage, and provides time for the ribosome to be correctly positioned at the ER translocon.

**159.** (A) Positively charged basic amino acids (Lys, Arg)
Proteins destined for the nucleus contain a short amino acid sequence called a Nuclear Localization Signal (NLS). These signals are typically characterized by a high concentration of basic, positively charged amino acids, such as lysine and arginine. The NLS can be located anywhere within the protein's primary structure and is not removed after transport, allowing the protein to re-enter the nucleus following each round of cell division.

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**160.** (D) Importin
Importins are specialized transport receptors that facilitate the movement of proteins into the nucleus. They recognize and bind to the Nuclear Localization Signal (NLS) on the "cargo" protein in the cytoplasm. Once the importin-cargo complex is formed, it interacts with the nuclear pore complex to move through the channel into the nucleus. Inside, the cargo is released, and the importin is recycled back to the cytoplasm for further transport cycles.

**161.** (A) 3 → 2 → 1 → 4
Mitochondrial protein import follows a strictly ordered pathway. It begins when the protein's presequence binds to an import receptor on the outer membrane. The protein is then threaded through the TOM complex in the outer membrane, followed by the TIM complex in the inner membrane. Once inside the matrix, the signal sequence is cleaved by a specialized peptidase, allowing the protein to fold into its final functional shape.

**162.** (D) COPII
Anterograde transport, the movement from the endoplasmic reticulum to the Golgi apparatus, is mediated by COPII-coated vesicles. The assembly of the COPII coat at ER exit sites is triggered by the Sar1 GTPase. This coat captures specific cargo proteins and curves the ER membrane to form a transport vesicle. Once the vesicle is released, the coat is shed, allowing the vesicle to dock and fuse with the cis-Golgi network.

**163.** (A) Endoplasmic reticulum lumen
Many proteins that function within the endoplasmic reticulum contain a specific C-terminal sequence, most commonly KDEL. This sequence acts as a retrieval signal. If an ER-resident protein is accidentally swept into a transport vesicle and carried to the Golgi, it is recognized by KDEL receptors. These receptors then package the "escaped" protein into COPI-coated vesicles for retrograde transport back to the ER, maintaining the organelle's protein composition.

**164.** (B) Mannose-6-phosphate
The mannose-6-phosphate (M6P) tag is the universal "address label" for lysosomal enzymes. This carbohydrate modification is added to specific mannose residues in the Golgi apparatus. M6P receptors in the trans-Golgi network bind to these tagged enzymes and concentrate them into clathrin-coated vesicles. These vesicles are then diverted to the late endosome and eventually the lysosome, ensuring that digestive enzymes are correctly delivered to their site of action.

**165.** (D) A C-terminal Ser-Lys-Leu (SKL) tripeptide
Most peroxisomal matrix proteins are targeted to the organelle by a specific signal known as Peroxisomal Targeting Signal 1 ($PTS_1$). This signal consists of a conserved tripeptide sequence, typically Serine-Lysine-Leucine (SKL), located at the extreme C-terminus of the protein. This sequence is recognized by a cytosolic receptor called Pex5, which then guides the protein to a translocon complex on the peroxisomal membrane for import.

**166.** (D) All mitochondrial proteins are coded by the mitochondrial genome and do not require import.
Although mitochondria possess their own genome, it only encodes a small fraction (about 1%) of the total proteins found in the organelle. The vast majority of mitochondrial proteins are encoded by the nuclear genome, synthesized on "free" ribosomes in the cytoplasm, and then actively imported into the mitochondria. Therefore, the claim that all mitochondrial proteins are produced locally and do not require import is a fundamental biological error.

**167.** (A) Ran GTPase
The directionality of nuclear transport is governed by the Ran GTPase, which exists in two distinct states. In the nucleus, Ran is kept in its GTP-bound form (Ran-GTP) by a specific exchange factor. In the cytoplasm, it is converted to Ran-GDP. This gradient ensures that transport receptors, such as importins and exportins, correctly bind or release their cargo depending on which side of the nuclear envelope they are currently located.

**168.** (D) The specific pairing of v-SNAREs on the vesicle with t-SNAREs on the target membrane
The accuracy of vesicular trafficking depends on a high level of specificity between the vesicle and its target. This is achieved by unique pairs of SNARE proteins. Each transport vesicle carries a specific V-SNARE that must match a corresponding T-SNARE on the target membrane. This molecular "lock and key"

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mechanism ensures that cargo from the ER only goes to the Golgi, and Golgi vesicles only fuse with their correct destinations.

**169.** (A) S phase
The S phase, or Synthesis phase, is the specific period during the eukaryotic cell cycle when the genomic DNA is replicated. This process ensures that every daughter cell will receive an exact copy of the genetic material. DNA replication is highly regulated to occur only once per cycle. Once S phase is complete, the cell possesses twice its normal amount of DNA and proceeds toward division in the subsequent phases.

**170.** (D) $G_0$ phase
The $G_0$ phase is a state of "quiescence" where cells exit the active cell cycle. Some cells, like mature neurons, enter $G_0$ permanently and never divide again. Others, like hepatocytes or stem cells, can remain in $G_0$ for long periods but are capable of re-entering the $G_1$ phase in response to specific growth factors or injury. This phase allows the cell to perform specialized functions without the metabolic burden of constant division.

**171.** (D) Cyclin-dependent kinases (CDKs)
Cyclin-dependent kinases (CDKs) are the master regulators of the cell cycle. These enzymes work by phosphorylating target proteins to drive the cell from one phase to the next. However, CDKs are inactive on their own and must bind to specific regulatory proteins called cyclins to function. The sequential activation and subsequent inactivation of various Cyclin-CDK complexes ensure that each stage of the cell cycle occurs in the correct order.

**172.** (B) Commit to DNA synthesis and complete the cell cycle
The $G_1$/S checkpoint, also known as the restriction point, is the most critical decision point in the cell cycle. Once a cell passes this threshold, it becomes irreversibly committed to replicating its DNA and completing the entire cycle, regardless of whether external growth factors are still present. At this stage, the cell carefully monitors its size, nutrient availability, and DNA integrity before proceeding to the synthesis phase.

**173.** (A) p53
Known as the "guardian of the genome," p53 is a tumor suppressor protein that prevents cells with damaged DNA from dividing. When DNA damage is detected at the $G_1$/S checkpoint, p53 levels rise and activate the transcription of p21, a CDK inhibitor. This halts the cell cycle, providing time for DNA repair. If the damage is too severe, p53 triggers apoptosis to prevent the transmission of mutations to daughter cells.

**174.** (D) Cyclin B and CDK1
The Maturation Promoting Factor (MPF) is the primary complex that triggers the transition from the $G_2$ phase into mitosis. It consists of two components: a regulatory subunit, Cyclin B, and a catalytic subunit, CDK1. The concentration of Cyclin B rises during $G_2$ and peaks in mitosis. Once activated, the MPF complex phosphorylates numerous target proteins, leading to nuclear envelope breakdown, chromosome condensation, and the formation of the mitotic spindle.

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**175.** (D) They possess intrinsic enzymatic kinase activity.
Cyclins are strictly regulatory proteins and do not have any enzymatic activity of their own. Their only function is to bind to and activate Cyclin-dependent kinases (CDKs). Once bound, the cyclin helps position the CDK near its target substrates and enhances its catalytic efficiency. Because cyclins lack intrinsic kinase activity, the claim that they can phosphorylate proteins independently is biologically incorrect.

**176.** (A) Both A and R are true and R is the correct explanation of A.
The Spindle Assembly Checkpoint (SAC) is a vital quality control mechanism during mitosis. Any kinetochore that is not properly attached to spindle microtubules generates a biochemical signal that inhibits the Anaphase-Promoting Complex (APC/C). This prevents the cell from entering anaphase prematurely. Only when all chromosomes are correctly aligned and attached does the inhibitory signal cease, allowing the APC/C to trigger the separation of sister chromatids and ensure equal genetic distribution.

**177.** (A) Securin
To initiate anaphase, the Anaphase-Promoting Complex (APC/C) targets the protein securin for degradation via the ubiquitin-proteasome pathway. Securin is an inhibitory protein that normally keeps the enzyme separase inactive. Once securin is destroyed, separase is freed to cleave the cohesin rings that hold the sister

chromatids together. This irreversible step allows the chromatids to be pulled apart toward opposite poles of the dividing cell.

**178.** (B) It releases the $E_2F$ transcription factor, enabling the transcription of S-phase genes.
The retinoblastoma (Rb) protein acts as a molecular "brake" on the cell cycle by binding to and inhibiting the $E_2F$ transcription factor. During the late $G_1$ phase, $G_1$-CDK complexes phosphorylate the Rb protein, causing it to change shape and release $E_2F$. Once free, $E_2F$ enters the nucleus and activates the transcription of genes necessary for DNA replication, effectively driving the cell from $G_1$ into the S phase.

**179.** (A) CDK1
The activation of the Maturation Promoting Factor (MPF) at the $G_2$/M transition is controlled by the phosphorylation state of its catalytic subunit, CDK1. The Wee1 kinase adds an inhibitory phosphate to CDK1, keeping it inactive. In response to DNA damage, the checkpoint pathway ensures this inhibitory phosphate is maintained, preventing the cell from entering mitosis with genomic errors. Only after repair can the Cdc25 phosphatase remove the phosphate and activate the complex.

**180.** (D) Cyclin-dependent kinase inhibitors (CKIs, e.g., p21, p27)
Cyclin-dependent kinase inhibitors (CKIs) are a family of proteins that provide a direct way to halt the cell cycle. These molecules, such as p21 and p27, bind physically to Cyclin-CDK complexes and block their enzymatic activity. By inhibiting these master regulators, CKIs allow the cell to pause the cycle in response to DNA damage, lack of growth factors, or environmental stress, ensuring that division only occurs under favorable conditions.

**181.** (C) Anaphase
Anaphase is the stage of mitosis characterized by the sudden separation of sister chromatids. Once the cohesin proteins holding them together are cleaved, the chromatids are pulled toward opposite poles of the cell by the shortening of kinetochore microtubules. This phase ensures that each new daughter cell will receive an identical and complete set of chromosomes, maintaining genetic stability across successive generations of cells.

**182.** (A) Prophase I
Crossing over is a fundamental genetic process that occurs exclusively during Prophase I of meiosis. During this extended stage, homologous chromosomes pair up and physically exchange segments of their DNA. This recombination creates new combinations of alleles on each chromosome, significantly increasing genetic diversity among the resulting gametes. This variability is a key driver of evolution and explains why offspring differ genetically from their parents.

**183.** (A) Pairing of homologous chromosomes (synapsis) during prophase I of meiosis
The synaptonemal complex is a ladder-like protein structure that forms between homologous chromosomes during the zygotene stage of Prophase I. Its primary role is to mediate synapsis, the precise longitudinal pairing of the homologues. By holding the chromosomes in close proximity, the synaptonemal complex provides the structural framework necessary for the subsequent process of crossing over and genetic recombination to occur accurately between non-sister chromatids.

**184.** (A) Centrosomes
In animal cells, the mitotic spindle is organized by two centrosomes that migrate to opposite poles of the cell during prophase. Each centrosome acts as a microtubule-organizing center, nucleating the formation of the various spindle fibers. These microtubules then grow toward the chromosomes and the cell equator, forming the complex machinery required to align and subsequently segregate the genetic material during the later stages of mitosis.

**185.** (D) Cell plate derived from Golgi vesicles fusing at the cell equator
Cytokinesis in plant cells is unique because the rigid cell wall prevents the cell from simply pinching in half. Instead, during telophase, the cell transports numerous Golgi-derived vesicles containing cell wall materials to the center of the dividing cell. These vesicles fuse to form a disc-like structure called the cell plate. The plate expands outward until it fuses with the original cell wall, creating a new partition between the two daughter cells.

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**186.** (A) P-3, Q-1, R-2, S-4
The cell cycle phases are defined by specific events. In mitotic metaphase, chromosomes align at the equator. Meiotic anaphase I is characterized by the separation of homologous chromosomes. During the diplotene stage of meiosis I, chiasmata become clearly visible as homologues begin to pull apart. Finally, in telophase, the nuclear envelope reforms around the newly separated genetic material at the poles. These steps ensure organized and accurate cell division.

**187.** (A) Four genetically unique haploid cells
Meiosis is a specialized form of cell division that reduces the chromosome number by half. It consists of two successive rounds of division (Meiosis I and II) following a single round of DNA replication. The end result of this process from one diploid progenitor cell is four haploid daughter cells. Due to the processes of crossing over and independent assortment, each of these gametes possesses a unique combination of genetic information.

**188.** (C) It physically holds the two sister chromatids together along their entire length.
The kinetochore is a specialized protein structure that assembles exclusively on the centromeric region of each chromosome. While it is essential for attaching chromosomes to spindle microtubules and facilitating their movement, it is not responsible for holding sister chromatids together along their entire length. That specific function is performed by cohesin proteins. Therefore, the statement claiming the kinetochore holds the chromatids together longitudinally is biologically inaccurate.

**189.** (A) Pachytene
Pachytene is the third sub-stage of Prophase I in meiosis. It is during this stage that the process of synapsis is complete, and homologous chromosomes are fully paired. The actual exchange of genetic material between non-sister chromatids, known as crossing over, occurs at this time. These crossover points are visible later in prophase as chiasmata, marking the physical legacy of the recombination event that occurred during pachytene.

**190.** (D) Polar (interpolar) microtubules
Polar microtubules, also known as interpolar microtubules, extend from the centrosomes but do not attach to chromosomes. Instead, they overlap with polar microtubules originating from the opposite pole in the center of the cell. During anaphase B, motor proteins push these overlapping microtubules apart while others promote their further elongation. This combined action exerts force that physically moves the two spindle poles further away from each other, helping to elongate the dividing cell.

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**191.** (A) Two $(n+1)$ and two $(n-1)$
Non-disjunction during Anaphase I occurs when a pair of homologous chromosomes fails to separate and instead moves together into one daughter cell. This error results in all four resulting gametes being abnormal. Two gametes will contain an extra chromosome $(n+1)$, while the other two will be missing a chromosome $(n-1)$. This condition, known as aneuploidy, can lead to genetic disorders such as Down syndrome if these gametes participate in fertilization.

**192.** (B) Separase
Separase is a cysteine protease that acts as the final trigger for the onset of anaphase. Throughout mitosis, sister chromatids are held together by a ring-like protein complex called cohesin. Once the spindle assembly checkpoint is satisfied, securin is degraded, and separase becomes active. Separase then cleaves the cohesin rings, allowing the sister chromatids to be pulled toward opposite poles, effectively initiating the process of chromosome segregation.

**193.** (B) Differentiate into any cell type of the adult organism, including extraembryonic tissues
Totipotency is the highest level of developmental potential. A totipotent cell, such as a zygote or a cell from the very earliest stages of an embryo, has the remarkable ability to divide and produce all the differentiated cells in an organism. Crucially, totipotent cells can also form extraembryonic tissues like the placenta. This means a single totipotent cell has the capacity to give rise to an entire, functional organism.

**194.** (D) Pluripotent
Pluripotent stem cells, such as those found in the inner cell mass of a blastocyst, have the ability to differentiate into any of the three germ layers: ectoderm, mesoderm, and endoderm. This means they can produce every cell type found in the adult body. However, unlike totipotent cells, they cannot form extraembryonic tissues like

the placenta and therefore cannot develop into a complete organism on their own.

**195.** (A) Multipotent
Multipotent stem cells are more specialized than pluripotent cells and are found in various tissues throughout the adult body. Their developmental potential is restricted to a specific family of related cell types. For example, hematopoietic stem cells in the bone marrow can differentiate into all the different kinds of blood cells (red cells, white cells, and platelets) but cannot form other tissue types like neurons or muscle cells.

**196.** (D) Transcription factors (e.g., Oct4, Sox2, c-Myc, Klf4)
Induced pluripotent stem (iPS) cells are created by "reprogramming" fully differentiated adult somatic cells, such as skin fibroblasts, back into an embryonic-like state. This breakthrough was achieved by Shinya Yamanaka using a specific cocktail of four transcription factors, now known as the Yamanaka factors. These proteins reset the cell's gene expression program, restoring the pluripotency and selF-renewal capabilities typical of embryonic stem cells.

**197.** (A) The differential expression of specific genes while maintaining a constant genome
Differentiation is the process by which a less specialized cell becomes more specialized. This change is not caused by the loss of DNA; instead, almost all cells in an organism contain the exact same genome. Differentiation occurs through differential gene expression, where specific sets of genes are turned on or off. This allows the cell to produce the unique proteins and structures necessary for its specialized role, such as a neuron or a muscle cell.

**198.** (C) Meristem
Meristems are specialized regions in plants containing undifferentiated cells that remain capable of continuous division. These tissues are responsible for the primary and secondary growth of the plant. Apical meristems at the tips of roots and shoots allow for elongation, while lateral meristems like the vascular cambium allow for increases in girth. Because these cells retain their embryonic character, they provide a constant source of new cells for the plant's entire life.

**199.** (A) Decreases
As a cell proceeds through the process of differentiation, it follows an increasingly narrow developmental path. A totipotent zygote becomes a pluripotent embryonic stem cell, which then becomes a multipotent progenitor, and finally a fully specialized, unipotent terminal cell. At each step, the cell commits to a specific lineage and loses the ability to form other cell types. Consequently, the overall developmental potential of the cell decreases as it matures.

**200.** (D) They exhibit a highly restricted and rapid rate of apoptosis to maintain numbers.
Stem cells are defined by their unique ability to selF-renew and differentiate. They often divide asymmetrically, producing one stem cell to maintain the population and one progenitor cell that will go on to specialize. Stem cells are not characterized by rapid or highly restricted apoptosis. In fact, many stem cell populations are remarkably long-lived and possess robust mechanisms to resist cell death and repair DNA damage, ensuring the long-term maintenance of the tissue.

**201.** (A) I only
Unlike animal cells, many differentiated plant cells retain the remarkable ability to revert to an undifferentiated state and eventually give rise to an entire new plant. This phenomenon, known as developmental plasticity or totipotency, is routinely exploited in plant tissue culture. Since many mature plant cells are not irreversibly committed to their specialized role, the statement claiming they lose totipotency permanently is incorrect. The other statements regarding pluripotency and unipotency are accurate.

**202.** (B) Hypertrophy
Hypertrophy refers to an increase in the total mass or size of a tissue or organ that results solely from the enlargement of its constituent individual cells. This type of growth occurs without an increase in the total number of cells through division. A classic example is the increase in skeletal muscle mass following exercise, where muscle fibers grow larger and produce more contractile proteins in response to physical demand.

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**203.** (A) DNA methylation of cytosine residues in promoter regions
Epigenetic modifications are chemical changes to DNA or histones that regulate gene expression without altering the underlying genetic sequence. A primary example is DNA methylation, where a methyl group is added to cytosine bases, typically in promoter regions. This modification often leads to gene silencing. These changes are stable and can be inherited by daughter cells, playing a vital role in maintaining the specialized identity of different cell types during differentiation.

**204.** (B) Forming concentration gradients that trigger different developmental programs
Morphogens are secreted signaling molecules that play a central role in pattern formation during development. They diffuse from a source, creating a concentration gradient across a field of cells. Cells respond differently depending on the concentration of the morphogen they receive; high levels may trigger one set of genes, while lower levels activate others. This positional information allows cells to adopt the correct identity relative to their location in the developing organism.

**205.** (C) Programmed, energy-dependent cellular suicide characterized by DNA fragmentation and membrane blebbing
Apoptosis is a highly regulated form of cell death used by multicellular organisms to remove unwanted, damaged, or potentially dangerous cells. Unlike traumatic cell death, apoptosis is an active, energy-requiring process. It follows a specific morphological pattern, including cell shrinkage, chromatin condensation, DNA fragmentation into precise segments, and the formation of membrane-enclosed "blebs" or apoptotic bodies. This orderly dismantling ensures that the cell's contents are not released into the surrounding tissue.

**206.** (D) Shrinkage of the cell and packaging into intact apoptotic bodies
The primary morphological difference between apoptosis and necrosis lies in the integrity of the cell. In apoptosis, the cell shrivels and its contents remain neatly packaged within membranes, forming apoptotic bodies that are rapidly consumed by neighboring cells or macrophages. In contrast, necrosis involves cellular swelling and the sudden rupture of the plasma membrane. This leakage of internal enzymes and inflammatory molecules into the surrounding environment causes significant tissue damage and inflammation.

**207.** (D) Caspases
Caspases are a family of specialized cysteine proteases that act as the central "executioners" of the apoptotic pathway. They are synthesized as inactive pro-enzymes and are activated through a cascade mechanism. Once active, caspases systematically cleave hundreds of target proteins, leading to the destruction of the nuclear lamina, the cytoskeleton, and the activation of DNases. This coordinated proteolytic activity results in the characteristic structural changes and eventual death of the apoptotic cell.

**208.** (B) Internal cellular stress, such as severe DNA damage or oxidative stress
The intrinsic, or mitochondrial, pathway of apoptosis is initiated from within the cell itself. It is triggered by various forms of severe internal stress, including irreparable DNA damage, extreme oxidative stress, or the loss of essential survival signals. These stimuli lead to the disruption of the mitochondrial membrane, initiating a cascade that eventually activates the executioner caspases. This pathway allows the cell to monitor its own health and selF-destruct if it becomes compromised.

**209.** (A) Cytochrome c
Cytochrome c is a small protein that normally resides in the mitochondrial intermembrane space, where it participates in the electron transport chain. During the initiation of the intrinsic apoptotic pathway, however, the outer mitochondrial membrane becomes permeable, allowing cytochrome c to escape into the cytosol. Once in the cytoplasm, it binds to the protein APAF-1 to form a wheel-like complex called the apoptosome, which then activates the initiator caspase-9.

**210.** (D) The BCl-2 family
The BCl-2 family of proteins acts as the primary gatekeeper of the intrinsic apoptotic pathway. This family includes both pro-apoptotic members (like Bax and Bak) that promote mitochondrial membrane permeability and anti-apoptotic members (like BCl-2 and BCl-xL) that prevent it. The balance between these opposing groups determines whether cytochrome c is released

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from the mitochondria, effectively deciding the fate of the cell in response to stress.

**211.** (A) I, II and IV
Apoptosis is regulated by specific molecular interactions. Bax and Bak are pro-apoptotic proteins that permeabilize mitochondria, while BCl-2 and BCl-xL act to prevent this process. The extrinsic pathway uses the DISC complex to initiate the death cascade from the surface. However, the apoptosome specifically activates caspase-9, not caspase-8. Therefore, while statements I, II, and IV are correct, the claim regarding the apoptosome's target is inaccurate.

**212.** (D) Fas receptor (CD95)
The extrinsic pathway of apoptosis is triggered by external signals binding to "death receptors" on the cell surface. These receptors are members of the tumor necrosis factor (TNF) receptor superfamily. The Fas receptor (CD95) is a classic example. When its ligand, FasL, binds to the receptor, it causes the intracellular domains to cluster and form a signaling platform that activates the initiator caspase-8, directly linking extracellular instructions to the cell's suicide machinery.

**213.** (A) Initiator caspases
Caspases are categorized based on their position in the apoptotic cascade. Initiator caspases, such as Caspase-8 and Caspase-9, are the first to be activated in response to death signals. Their primary role is to process and activate the "executioner" caspases (like Caspase-3 and Caspase-7). This hierarchical organization allows for signal amplification and provides multiple points where the cell can regulate the decision to proceed with programmed cell death.

**214.** (A) Cleavage of DNA between nucleosomes by Caspase-Activated DNase (CAD)
A hallmark of apoptosis is the systematic fragmentation of genomic DNA. Executioner caspases activate an enzyme called Caspase-Activated DNase (CAD) by cleaving its inhibitor. CAD then cuts the DNA at the accessible linker regions between nucleosomes. Because nucleosomes are spaced at regular intervals of approximately 180 to 200 base pairs, this process produces DNA fragments of discrete, varying lengths that appear as a characteristic "ladder" pattern when analyzed by gel electrophoresis.

**215.** (C) Phosphatidylserine
In healthy cells, the phospholipid phosphatidylserine is strictly confined to the inner leaflet of the plasma membrane. During the early stages of apoptosis, however, the enzymes that maintain this asymmetry are inactivated, and "scramblases" move the lipid to the outer surface. This exposure of phosphatidylserine acts as a powerful "eat-me" signal. It is recognized by receptors on phagocytic cells, ensuring that apoptotic bodies are consumed before they can rupture and cause inflammation.

**216.** (D) DNA cleavage in necrosis is highly specific and internucleosomal, unlike apoptosis.
DNA degradation in necrosis is a disorganized and random process caused by the leakage of various lysosomal enzymes after the cell's structural integrity fails. This results in a "smear" of DNA fragments rather than the precise, internucleosomal "laddering" seen in apoptosis. Therefore, the statement claiming that necrotic DNA cleavage is highly specific is biologically incorrect. The other comparisons regarding ATP requirements, inflammation, and cellular distribution are accurate.

**217.** (A) Autocrine signaling
Autocrine signaling occurs when a cell secretes signaling molecules that bind to receptors on its own plasma membrane or that of similar neighboring cells. This type of communication allows a cell to regulate its own behavior or coordinate a group response among identical cells. It is particularly common during development, where it helps reinforce cell identity, and in the immune system, where it can stimulate rapid cell proliferation.

**218.** (D) Travel through the bloodstream to act on distant target cells
Endocrine signaling is the mechanism used for long-distance communication within the body. Specialized endocrine glands secrete signaling molecules, known as hormones, directly into the circulatory system. These hormones travel through the bloodstream, allowing them to reach and influence target cells located in distant organs and tissues. This system is essential for coordinating complex physiological processes such as metabolism, growth, and reproduction across the entire organism.

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**219.** (A) Paracrine signaling
Paracrine signaling involves the release of signaling molecules that act only on cells in the immediate vicinity of the signaling cell. Synaptic transmission is a highly specialized and rapid form of paracrine signaling. Here, a neuron releases neurotransmitters into a narrow space called the synaptic cleft. The molecules only need to diffuse a very short distance to reach the receptors on the adjacent target cell, ensuring precise and localized communication.

**220.** (D) A higher affinity of the receptor for the ligand
The dissociation constant ($K_d$) represents the concentration of ligand at which half of the receptor binding sites are occupied at equilibrium. There is an inverse relationship between $K_d$ and binding affinity. A lower $K_d$ value means that the receptor and ligand bind more tightly to each other, and a lower concentration of the ligand is sufficient to achieve significant receptor activation. This value is a fundamental measure of the strength of molecular interactions.

**221.** (A) I and III
Cell signaling is governed by specific physical and chemical principles. Hydrophilic ligands cannot cross the lipid bilayer and therefore must bind to extracellular receptors on the cell surface. Furthermore, agonists are molecules that bind to and activate a receptor, whereas antagonists are the ones that inhibit it. Therefore, the statements claiming hydrophilic ligands bind intracellularly and defining agonists as inhibitors are both incorrect in the context of cell signaling.

**222.** (C) Receptor desensitization or downtime
To prevent overstimulation and maintain sensitivity to new signals, cells utilize various mechanisms to terminate or dampen a signaling response. One common method is receptor desensitization, where prolonged exposure to a ligand leads to a decrease in the receptor's responsiveness. This can occur through chemical modification of the receptor, its temporary internalization into vesicles, or its total degradation by lysosomes, allowing the cell to "reset" and respond appropriately to future signals.

**223.** (D) It requires direct physical contact between the signaling cell and the target cell.
Juxtacrine signaling, also known as contact-dependent signaling, involves communication between cells that are physically touching. In this mechanism, the signaling molecule is not secreted; instead, it remains bound to the plasma membrane of the signaling cell. The message is transmitted when this membrane-bound ligand interacts directly with a receptor on the surface of an adjacent cell. This type of signaling is critical for controlling cell fate decisions during embryonic development.

**224.** (A) Antagonist
An antagonist is a type of ligand that binds to a specific receptor with high affinity but fails to trigger a biological response. By occupying the receptor's binding site, the antagonist physically blocks the endogenous signaling molecule from binding and activating the receptor. This pharmacological property is widely used in medicine to inhibit overactive signaling pathways, such as using beta-blockers to manage heart rate and blood pressure.

**225.** (B) Is a gas that diffuses directly across membranes to activate intracellular enzymes.
Nitric oxide (NO) is a short-lived gaseous signaling molecule produced by various cells. Because it is small and non-polar, it can pass freely through the plasma membrane of nearby cells without the need for a surface receptor. Once inside the target cell, NO typically binds to and activates the enzyme soluble guanylate cyclase, leading to the production of cGMP. This process is essential for regulating blood flow and smooth muscle relaxation.

**226.** (D) The interaction and integration of different signaling pathways within a cell.
In biological systems, signaling pathways do not operate in total isolation. Instead, they form complex networks where components from one pathway can influence the activity of another. This "cross-talk" allows the cell to integrate information from multiple external signals and produce a coordinated, appropriate response. It ensures that the final cellular output, such as growth or differentiation, is based on the overall physiological context rather than a single isolated instruction.

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**227.** (A) It must possess a specific ligand-binding site and undergo a conformational change upon binding.
For a molecule to function as a receptor, it must satisfy two fundamental requirements. First, it must have a high-affinity binding site that is structurally specific for a particular signaling molecule. Second, upon binding the ligand, the receptor must undergo a physical change in its shape or conformation. This conformational change is the "switch" that transmits the signal from the exterior of the cell to its internal molecular machinery.

**228.** (C) Signal amplification (cascade)
Signal amplification is a hallmark of most signal transduction pathways. It occurs when a single activated receptor molecule triggers the activation of multiple downstream effector molecules, such as enzymes or second messengers. These effectors, in turn, activate even more molecules in the next step of the pathway. This "multiplier effect" ensures that a very small extracellular signal can result in a massive and rapid physiological response within the target cell.

**229.** (C) They span the plasma membrane seven times (7-TM domain).
G-protein coupled receptors (GPCRs) represent the largest and most diverse family of cell-surface receptors in eukaryotes. Despite their varied ligands, they all share a characteristic structural motif: a single polypeptide chain that winds back and forth across the plasma membrane seven times. This 7-transmembrane (7-TM) architecture is essential for their function, as it links the extracellular ligand-binding domain to the intracellular regions that interact with heterotrimeric G-proteins.

**230.** (D) The alpha subunit exchanges its bound GDP for GTP.
In the resting state, the G-protein alpha subunit is bound to GDP and is associated with the beta-gamma complex. When a ligand binds to the GPCR, the receptor undergoes a conformational change that acts as a guanine nucleotide exchange factor (GEF) for the G-protein. This causes the alpha subunit to release its GDP and bind a new molecule of GTP. This exchange triggers the dissociation of the G-protein into two active signaling components.

**231.** (A) Receptor dimerization and subsequent auto-cross-phosphorylation
Receptor Tyrosine Kinases (RTKs) are typically single-pass transmembrane proteins that exist as monomers in the absence of a signal. Ligand binding induces two receptor monomers to come together to form a dimer. This proximity allows the kinase domains on the intracellular tails to phosphorylate each other on specific tyrosine residues. These phosphorylated tyrosines then serve as high-affinity docking sites for various intracellular signaling proteins, initiating the downstream response.

**232.** (D) Small monomeric GTPases
Ras is a member of a large family of small, monomeric GTPase proteins that are anchored to the cytosolic face of the plasma membrane. Unlike the heterotrimeric G-proteins associated with GPCRs, Ras acts as a single-unit molecular switch. It cycles between an inactive GDP-bound state and an active GTP-bound state. In RTK signaling, Ras is activated by exchange factors and subsequently triggers the MAP kinase cascade, which is fundamental for controlling cell growth.

**233.** (B) MAPKKK → MAPKK → MAPK
The MAP kinase signaling pathway is organized into a strictly conserved three-tiered cascade. It begins with the activation of a MAP kinase kinase kinase (e.g., Raf). This enzyme phosphorylates and activates a MAP kinase kinase (e.g., MEK). Finally, the MAPKK phosphorylates and activates the MAP kinase (e.g., ERK). This sequential organization provides multiple levels for signal regulation and massive amplification of the initial stimulus before it reaches the nucleus.

**234.** (C) GPCRs directly phosphorylate target proteins in the cytosol.
G-protein coupled receptors do not possess intrinsic enzymatic activity. Instead, they function by activating membrane-bound G-proteins, which then interact with and regulate other effector enzymes (like adenylate cyclase) or ion channels. These effectors subsequently produce second messengers that trigger the internal response. Therefore, the statement claiming that GPCRs directly phosphorylate proteins in the cytosol is incorrect, as phosphorylation is typically handled by downstream kinases like PKA or PKC.

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**235.** (A) $SH_2$ domain
$SH_2$ (Src Homology 2) domains are specialized protein motifs found in many intracellular signaling and adaptor proteins. These domains are specifically evolved to recognize and bind to phosphorylated tyrosine residues within a particular amino acid context. By utilizing $SH_2$ domains, signaling molecules like Grb2 can be recruited to the tails of activated Receptor Tyrosine Kinases, effectively linking the cell surface receptor to downstream pathways like the Ras-MAPK cascade.

**236.** (A) Both A and R are true and R is the correct explanation of A.
Cholera toxin is produced by the bacterium Vibrio cholerae. The toxin enters intestinal epithelial cells and chemically modifies the Gs-alpha subunit by adding an ADP-ribose group. This modification permanently "locks" the G-protein in its active, GTP-bound state by destroying its ability to hydrolyze GTP. The resulting continuous activation of adenylate cyclase leads to a massive surge in cAMP, causing the cells to expel chloride and water, resulting in life-threatening diarrhea.

**237.** (A) Receptor Tyrosine Kinase (RTK)
The insulin receptor belongs to the family of Receptor Tyrosine Kinases. It is a pre-formed dimer consisting of two alpha and two beta subunits. When insulin binds to the extracellular alpha subunits, it triggers the auto-phosphorylation of tyrosine residues on the intracellular beta subunits. This activation creates docking sites for insulin receptor substrates (IRS), which then initiate complex signaling pathways that regulate glucose uptake, glycogen synthesis, and cell growth throughout the body.

**238.** (D) They bind multiple components of a kinase cascade to ensure efficiency and specificity.
Scaffolding proteins act as molecular "organizers" in signal transduction. By physically binding to several sequential enzymes in a pathway, such as the three kinases of the MAPK cascade, they hold them in close proximity. This arrangement ensures that the kinases react specifically with each other and not with other signaling proteins. Scaffolding increases the speed of signal transmission and prevents "cross-talk" between different pathways that might use similar components.

**239.** (A) Protein phosphatase
To prevent continuous signaling and ensure that the cellular response is transient, the phosphate groups added during the MAPK cascade must be removed. This task is performed by protein phosphatases. These enzymes specifically dephosphorylate the active kinases, returning them to their inactive state. The balance between the activity of upstream kinases and these downstream phosphatases determines the duration and intensity of the MAP kinase signal within the cell.

**240.** (D) IP3 and DAG
Activation of the Gq family of G-proteins leads to the stimulation of the membrane-bound enzyme phospholipase C-beta (PLC-beta). This enzyme catalyzes the cleavage of the phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2) into two distinct second messengers: inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). These two molecules then diverge to initiate different branches of the intracellular signaling cascade, ultimately regulating calcium levels and protein kinase activity.

**241.** (B) Adenylate cyclase
Adenylate cyclase is a large, multi-pass transmembrane enzyme that plays a central role in GPCR signaling. When stimulated by the activated Gs-alpha subunit, adenylate cyclase converts molecules of ATP into cyclic AMP (cAMP). This reaction involves the removal of two phosphate groups and the formation of a cyclic bond. The resulting cAMP acts as a ubiquitous second messenger, relaying the signal from the plasma membrane to various targets throughout the cytoplasm.

**242.** (B) Protein Kinase A (PKA)
In most eukaryotic cells, the primary target of cyclic AMP is Protein Kinase A (PKA), also known as cAMP-dependent protein kinase. PKA is a tetramer consisting of two regulatory and two catalytic subunits. When cAMP levels rise, it binds to the regulatory subunits, causing them to dissociate and release the active catalytic subunits. These free subunits then phosphorylate specific serine and threonine residues on diverse target proteins to alter their activity.

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**243.** (B) Hydrolysis of cAMP to 5'-AMP by phosphodiesterase
To ensure that signaling is sharp and reversible, the cell must rapidly eliminate second messengers once the stimulus is gone. For cAMP, this is achieved by a family of enzymes called cyclic nucleotide phosphodiesterases. These enzymes break the cyclic bond in cAMP, converting it into inactive 5'-adenosine monophosphate (5'-AMP). By continuously degrading cAMP, phosphodiesterases ensure that the concentration of the messenger only remains high as long as adenylate cyclase is actively producing it.

**244.** (A) Diacylglycerol (DAG) and Inositol 1,4,5-trisphosphate (IP3)
Phospholipase C (PLC) is an effector enzyme that acts on the specialized membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP2). Upon activation, PLC hydrolyzes the bond between the glycerol and the phosphate group of the lipid. This results in the production of two important second messengers: IP3, which is water-soluble and diffuses into the cytosol, and DAG, which is hydrophobic and remains embedded in the plasma membrane.

**245.** (B) It binds to receptors on the smooth ER, causing the release of stored $Ca^{2+}$ into the cytosol.
Once generated by the cleavage of PIP2, inositol 1,4,5-trisphosphate (IP3) diffuses through the cytosol to the smooth endoplasmic reticulum. Here, it binds to specific IP3-gated calcium channels. Upon binding, these channels open, allowing calcium ions stored in the ER lumen to flood into the cytoplasm. This sudden increase in cytosolic calcium concentration acts as a powerful signal that triggers various cellular processes, including muscle contraction, secretion, and metabolism.

**246.** (A) P-3, Q-1, R-4, S-2
Second messengers activate specific target proteins to relay signals. Cyclic AMP (cAMP) is the primary activator of Protein Kinase A, while cyclic GMP (cGMP) targets Protein Kinase G. Diacylglycerol (DAG) remains in the membrane to activate Protein Kinase C. Finally, calcium ions function by binding to and activating the regulatory protein calmodulin. This coordinated system allows a wide variety of extracellular signals to be translated into specific intracellular responses.

**247.** (A) Visual phototransduction cascade in rod cells of the retina
Cyclic GMP (cGMP) is a crucial second messenger in several specialized systems, most notably in the retina. In the dark, high levels of cGMP keep cation channels open in rod cells. When light hits the photopigment rhodopsin, it triggers a signaling cascade that activates a phosphodiesterase, which rapidly degrades cGMP. The resulting drop in cGMP levels causes the ion channels to close, hyperpolarizing the cell and initiating the visual signal to the brain.

**248.** (C) $Ca^{2+}$
Diacylglycerol (DAG) is generated at the plasma membrane and recruits the enzyme Protein Kinase C (PKC) from the cytosol. However, for most isoforms of PKC to become fully active, they must also bind to calcium ions. This dual requirement for both DAG and $Ca^{2+}$ means that PKC activation is typically linked to signaling pathways that stimulate both Phospholipase C and the release of calcium from the endoplasmic reticulum.

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**249.** (D) The resting cytosolic concentration of $Ca^{2+}$ is kept extremely high.
For calcium to function effectively as a second messenger, its resting concentration in the cytosol must be kept extremely low, typically around 100 nanomolar. This is achieved by active transport pumps that move calcium out of the cell or into the endoplasmic reticulum and mitochondria. Because the resting level is so low, even a small release of calcium from storage creates a massive relative increase that the cell can easily detect.

**250.** (B) It wraps around and activates various target proteins, such as CaM-kinases.
Calmodulin is a small, highly conserved protein that acts as a universal calcium sensor. It possesses four high-affinity binding sites for calcium. When cytosolic calcium levels rise, calmodulin binds the ions and undergoes a dramatic conformational change. The resulting $Ca^{2+}$/calmodulin complex can then bind to and regulate the activity of many different target proteins, including CaM-kinases, which go on to phosphorylate other proteins and mediate the cellular response to the calcium signal.

**251.** (B) Nitric oxide (NO)
Nitric oxide is a unique paracrine signaling molecule that travels directly through cell membranes. In smooth muscle cells, NO binds to and activates the enzyme soluble guanylate cyclase. This enzyme then converts GTP into cyclic GMP (cGMP). The increase in cGMP activates Protein Kinase G, which leads to the phosphorylation of proteins that decrease cytosolic calcium and promote muscle relaxation. This mechanism is central to the regulation of blood pressure and vascular tone.

**252.** (A) A single ligand molecule can ultimately activate thousands of effector proteins.
The primary advantage of a second messenger cascade is the massive amplification of the original signal. Because enzymes like adenylate cyclase or various kinases can catalyze many reactions while active, a single extracellular signaling molecule can lead to the production of thousands of second messengers. These messengers then activate thousands of downstream kinases, resulting in a robust and widespread cellular response even when the initial stimulus is present at very low concentrations.

**253.** (A) Small size and hydrophobicity to cross the plasma membrane
Intracellular receptors respond to signaling molecules that can pass directly through the lipid bilayer of the cell. For this reason, their ligands must be relatively small and highly hydrophobic (lipid-soluble). This class of molecules includes steroid hormones, thyroid hormones, and certain vitamins like Vitamin D. Because these ligands can diffuse freely into the cell, they do not require a cell-surface receptor to transmit their message.

**254.** (B) Cortisol, Estrogen, and Testosterone
The steroid receptor superfamily is a group of transcription factors that are activated by lipid-soluble hormones derived from cholesterol. This group includes receptors for the glucocorticoid cortisol, as well as sex hormones like estrogen, progesterone, and testosterone. Because these hormones are hydrophobic, they travel through the blood bound to carrier proteins and then diffuse directly into target cells to meet their specific receptors in the cytoplasm or nucleus.

**255.** (A) Ligand-dependent transcription factor
Intracellular steroid receptors function as ligand-dependent transcription factors. In their inactive state, they cannot bind to DNA effectively. However, when the specific hormone binds to the receptor, it triggers a conformational change that "activates" the protein. The ligand-receptor complex then moves to the nucleus and binds to specific DNA sequences to regulate the transcription of target genes. This allows hormones to directly control the synthesis of new proteins within the cell.

**256.** (C) Zinc-finger DNA-binding domain
All members of the steroid receptor superfamily share a highly conserved central domain responsible for DNA binding. This domain contains two "zinc-finger" motifs, where a zinc ion is coordinated by four cysteine residues. These motifs allow the receptor to physically interact with and recognize specific nucleotide sequences in the promoter regions of target genes. This structural feature is essential for the receptor to act as a precise regulator of gene expression.

**257.** (A) Hsp90 (Heat shock protein 90)
In the absence of their hormone ligands, many steroid receptors, such as the glucocorticoid receptor, are kept in an inactive state within the cytosol. They are physically bound to a complex of chaperone proteins, most notably Hsp90. These chaperones help maintain the receptor in a conformation that is ready to bind the hormone but prevents it from entering the nucleus or binding to DNA prematurely. Upon hormone binding, the chaperones are released.

**258.** (B) The receptor undergoes a conformational change, releases chaperones, and translocates to the nucleus.
Hormone binding is the "trigger" that activates an intracellular receptor. The binding of the steroid molecule causes the receptor to change its shape, which results in the dissociation of inhibitory chaperone proteins like Hsp90. This conformational shift also exposes a Nuclear Localization Signal (NLS) on the receptor. Consequently, the activated hormone-receptor complex is rapidly transported through nuclear pore complexes into the nucleus, where it can begin its work of regulating gene transcription.

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**259.** (B) Specific DNA sequences in the promoter/ enhancer regions of target genes where the receptor binds
Hormone Response Elements (HREs) are short, specific sequences of DNA located within the regulatory regions of target genes. Each type of steroid receptor recognizes and binds to a specific HRE. For example, the estrogen receptor binds to Estrogen Response Elements. The binding of the activated hormone-receptor complex to these DNA sequences is the final step that allows the hormone to either stimulate or repress the transcription of that particular gene.

**260.** (C) Bound to DNA in the nucleus, often repressing transcription
Unlike many steroid receptors that wait in the cytosol, thyroid hormone receptors are typically found already located within the nucleus, bound to their specific Hormone Response Elements on the DNA. In the absence of the hormone ($T_3$ or $T_4$), these receptors often act as transcriptional repressors by recruiting co-repressor proteins. When the thyroid hormone enters the nucleus and binds to the receptor, it causes a conformational change that replaces the repressors with activators.

**261.** (A) They are large and/or hydrophilic, thus incapable of diffusing across the lipid bilayer.
Peptide hormones are composed of amino acid chains and are generally large and water-soluble (hydrophilic). Because of these physical properties, they cannot penetrate the hydrophobic core of the plasma membrane. To transmit their signal, they must bind to receptors on the outer surface of the cell. These surface receptors then activate intracellular signaling cascades or second messenger systems to relay the message to the cell's interior.

**262.** (B) The physiological response they induce is typically much faster (milliseconds) than that of ion channel receptors.
Signaling via intracellular receptors involves changes in gene expression and the synthesis of new proteins. This process is inherently slow, typically taking minutes to hours or even days to produce a physiological effect. In contrast, ion channel receptors can change the membrane potential in milliseconds. Therefore, the statement claiming that intracellular receptor responses are faster than those of ion channels is biologically incorrect. The other statements regarding genomic action and ligands are accurate.

**263.** (A) Genomic action
The primary and most well-studied mechanism of steroid hormone action is termed "genomic action." This refers to the hormone's ability to enter the cell, bind to an intracellular receptor, and directly regulate the transcription of specific genes in the nucleus. Because this process results in the production of new messenger RNA and subsequent protein synthesis, it represents the permanent or long-term structural and functional changes induced by the hormone.

**264.** (C) They act as an antagonist in some tissues (e.g., breast) but an agonist in others (e.g., bone).
Selective Estrogen Receptor Modulators (SERMs) are unique compounds that interact with the estrogen receptor but produce different effects depending on the tissue type. For example, tamoxifen acts as an antagonist in breast tissue, blocking estrogen's growth-promoting effects, which is useful in treating cancer. However, it can act as an agonist in bone, helping to maintain density. This tissue-specific behavior is due to the different co-activator proteins present in various cells.

**265.** (B) Synapse
A synapse is a specialized junction that allows a neuron to pass an electrical or chemical signal to another cell. In a chemical synapse, the two cells are separated by a tiny gap called the synaptic cleft. The signal is transmitted when the presynaptic neuron releases neurotransmitters that diffuse across the gap and bind to receptors on the postsynaptic target cell, ensuring one-way communication and providing a site for signal processing and modulation.

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**266.** (D) $Ca^{2+}$
When an action potential reaches the presynaptic terminal of a neuron, it triggers the opening of voltage-gated calcium channels. The resulting rapid influx of calcium ions into the cytosol is the essential signal for neurotransmitter release. The rise in calcium causes synaptic vesicles to fuse with the presynaptic membrane, discharging their contents into the synaptic cleft. This calcium-dependent exocytosis is a fundamental step in all chemical synaptic transmission.

**267.** (A) Directly opening an ion pore upon binding a specific neurotransmitter
Ligand-gated ion channels, also known as ionotropic receptors, are specialized transmembrane proteins that combine a receptor and an ion channel into a single complex. When a specific neurotransmitter binds to the extracellular part of the protein, it causes an immediate conformational change that opens a central pore. This allows specific ions to flow down their electrochemical gradient, rapidly changing the membrane potential of the postsynaptic cell and transmitting the signal.

**268.** (C) Ligand-gated cation channel
The nicotinic acetylcholine receptor is a well-studied example of a ligand-gated ion channel found at the neuromuscular junction. It is composed of five subunits that form a central pore. When acetylcholine binds to the receptor, the pore opens and becomes permeable to small cations, primarily sodium and potassium. The resulting influx of sodium ions depolarizes the muscle cell membrane, which eventually triggers the contraction of the muscle fiber.

**269.** (C) They allow direct electrical and metabolic coupling by passing small ions and molecules between cells.
Gap junctions are specialized intercellular connections that create direct channels between the cytoplasm of adjacent animal cells. These channels allow the free passage of small ions, metabolites, and second messengers like cAMP or calcium. By enabling this direct exchange, gap junctions facilitate electrical and metabolic coupling. This is essential for the synchronized contraction of cardiac muscle cells and the coordinated response of cells within a tissue to various stimuli.

**270.** (C) 6
A gap junction is formed by the alignment of two hemichannels, called connexons, from neighboring cells. Each connexon is a cylindrical structure composed of six individual protein subunits known as connexins. These six subunits arrange themselves around a central aqueous pore. When two connexons from adjacent cells meet and dock in the extracellular space, they create a continuous, regulated channel that allows for direct communication between the two cytoplasms.

**271.** (C) Plant cells
Plasmodesmata are the primary intercellular junctions found in plant cells. Because plants have a rigid cell wall, they cannot use the same types of junctions found in animals. Plasmodesmata are narrow channels that penetrate through the cell walls of adjacent plant cells, physically connecting their cytoplasms. This creates a continuous living network called the symplast, which allows for the transport of water, nutrients, and signaling molecules throughout the entire plant body.

**272.** (A) Smooth Endoplasmic Reticulum
Each plasmodesma contains a narrow, cylindrical structure called a desmotubule running through its center. The desmotubule is a specialized extension of the smooth endoplasmic reticulum that is continuous between the two connected plant cells. The narrow space between the desmotubule and the plasma membrane, known as the cytoplasmic sleeve, is where most of the regulated transport of ions and small molecules occurs, facilitating communication through the symplast.

**273.** (B) Provide strong mechanical adhesion between adjacent cells to resist shearing forces
Desmosomes are specialized anchoring junctions that act like "spot welds" between adjacent cells. They are particularly abundant in tissues that are subject to significant mechanical stress, such as the skin and the heart. By physically linking the cytoskeletons of neighboring cells, desmosomes distribute mechanical forces across the entire tissue layer. This prevents the cells from pulling apart and ensures the structural integrity of the organ during stretching or contraction.

**274.** (C) Intermediate filaments (e.g., keratin)
The strength of a desmosome comes from its connection to the cell's internal framework. On the cytosolic side of each desmosome is a dense protein plaque. This plaque serves as an anchoring site for intermediate filaments, such as keratin in epithelial cells. By linking these tough, rope-like filaments from one cell to the next, desmosomes create a continuous structural network that provides immense mechanical resilience to the entire tissue.

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**275.** (C) The process strictly operates in a bidirectional manner across the same chemical synapse.
Chemical synaptic transmission is inherently unidirectional. Neurotransmitters are stored and released only from the presynaptic terminal, and the specific receptors for these molecules are located on the postsynaptic membrane. This one-way flow of information ensures that nerve impulses travel in a defined direction through a neural circuit. Therefore, the statement claiming that chemical synapses operate in a strictly bidirectional manner is a fundamental misconception in neurobiology.

**276.** (B) Prevent the leakage of solutes and water between the cells (paracellular pathway)
Tight junctions are the uppermost junctions in epithelial cell layers, forming a continuous seal around the circumference of each cell. Their primary function is to block the paracellular pathway, preventing the uncontrolled movement of water, ions, and molecules through the spaces between cells. This allows epithelial sheets, such as the lining of the gut or the blood-brain barrier, to act as highly selective and effective physiological barriers.

**277.** (A) Cell lysis or homogenization
Subcellular analysis begins with the controlled disruption of the plasma membrane, a process known as cell lysis or homogenization. The goal is to break the outer boundary to release the internal components while using gentle enough conditions to keep the organelles, such as mitochondria and the nucleus, intact and functional. The resulting mixture of cellular parts, known as the homogenate, can then be further separated into its individual components.

**278.** (A) Dounce homogenization
Dounce homogenization is a gentle mechanical method for breaking open cells. It involves using a glass mortar and a precisely fitting pestle. As the pestle is moved, the cells are forced through a narrow space, creating shear forces that rupture the plasma membrane without damaging the more robust internal organelles. This technique is typically performed on ice to prevent heat buildup, ensuring that enzymes and proteins remain stable during the process.

**279.** (D) Size and density
Differential centrifugation is a fundamental technique for purifying organelles from a cell homogenate. It works by spinning the mixture at a series of increasingly higher speeds. Larger and denser components, such as nuclei, settle to the bottom as a "pellet" more quickly and at lower centrifugal forces. By sequentially removing these pellets and spinning the remaining liquid (supernatant) at higher speeds, researchers can separate cellular components based on their physical size and density.

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**280.** (C) Nuclei and unbroken cells
In a standard differential centrifugation procedure, the first step involves a low-speed spin, typically around 600 to 1000 x g. Because the nucleus is the largest and most massive organelle in the eukaryotic cell, it experiences the greatest centrifugal force. Consequently, nuclei, along with any cells that were not successfully broken during homogenization, will be the first components to sediment and form a pellet at the bottom of the tube.

**281.** (D) Mitochondria, chloroplasts, and peroxisomes
After the nuclei have been removed, the remaining supernatant is subjected to a second, moderate-speed centrifugation, often around 15,000 x g. This force is sufficient to pellet "medium-sized" organelles. This fraction, sometimes called the large-particle fraction, primarily contains mitochondria, chloroplasts (in plant cells), and peroxisomes. By isolating this pellet, scientists can study the specific metabolic activities and protein compositions of these energy-converting and oxidative organelles.

**282.** (A) Isopycnic (density-gradient) ultracentrifugation
When organelles have very similar sizes, they cannot be separated by differential centrifugation because they will pellet at the same speed. In these cases, researchers use isopycnic (equilibrium) density-gradient centrifugation. This technique separates components solely based on their buoyant density. The sample is layered on a gradient of a dense substance like sucrose. During high-speed spinning, each organelle migrates until it reaches a point where its own density matches that of the surrounding medium.

**283.** (B) A position where its density perfectly matches the density of the surrounding gradient
In isopycnic ultracentrifugation, the term "isopycnic" means "same density." The centrifuge

tube contains a pre-formed gradient of increasing density from top to bottom. When spun at very high speeds, organelles will move through the gradient until they reach their "equilibrium position." At this point, the buoyant force exactly balances the centrifugal force because the organelle's density is equal to the density of the gradient, causing it to float as a distinct band.

**284.** (A) Rapidly analyze physical and chemical characteristics of single cells as they flow in a fluid stream through a laser
Flow cytometry is a high-throughput technology used to analyze individual cells in a heterogeneous population. Cells are suspended in a fluid and passed one-by-one through a focused laser beam. As each cell intercepts the light, it scatters the beam and may emit fluorescence if it has been labeled. Specialized detectors capture these signals, providing quantitative data on thousands of cells per second regarding their size, complexity, and protein expression.

**285.** (D) Relative size or volume
In a flow cytometer, forward scatter (FSC) refers to the light that is deflected at a small angle as it passes around a cell. The amount of light scattered in this direction is generally proportional to the cross-sectional area of the cell. Therefore, the FSC signal is used by researchers as a reliable indicator of the relative size or volume of each individual cell within the sample being analyzed.

**286.** (A) Internal complexity and granularity
Side scatter (SSC) in flow cytometry refers to the light that is reflected or refracted at a 90-degree angle from the laser's path. This scatter is caused by internal structures within the cell, such as the nucleus, granules, and various organelles. A high SSC signal indicates a cell with high internal complexity or granularity, such as a neutrophil, while a low SSC signal characterizes cells with simpler internal structures, like lymphocytes.

**287.** (A) The ability to physically separate and collect distinct cell subpopulations based on their fluorescent signals
Fluorescence-Activated Cell Sorting (FACS) is a specialized application of flow cytometry. Beyond just analyzing the cells, a FACS instrument can use the detected signals to make a split-second decision about each cell. It can then apply an electrical charge to the droplet containing that cell, allowing it to be deflected into a separate collection tube. This allows scientists to isolate highly pure populations of specific cells for further cultivation or experimentation.

**288.** (A) Propidium Iodide (PI)
Propidium iodide is a fluorescent dye that binds stoichiometrically to DNA by intercalating between the bases. Because it cannot pass through intact membranes, it is used on fixed or permeabilized cells. In flow cytometry, the intensity of the PI fluorescence is directly proportional to the amount of DNA in the cell. This allows researchers to distinguish between cells in different phases of the cell cycle ($G_1$, S, or $G_2$/M) based on their DNA content.

**289.** (D) Differences in refractive index and thickness within cellular structures converted into differences in light intensity
Most living cells are transparent and show very little contrast under a standard brightfield microscope. Phase-contrast microscopy solves this by exploiting the fact that light travels at different speeds through various parts of the cell due to differences in density and refractive index. The microscope's optical system converts these invisible phase shifts into visible variations in brightness, allowing scientists to see the internal details of living, unstained cells in high detail.

**290.** (B) Illuminating the specimen with light of a specific excitation wavelength and capturing the emitted light of a longer wavelength
Fluorescence microscopy relies on the unique properties of fluorophores. The specimen is illuminated with high-energy light at a specific "excitation" wavelength. The fluorophores in the sample absorb this energy and almost immediately emit light at a lower-energy, longer "emission" wavelength. By using filters to block the excitation light and only capture the emitted light, the microscope creates a bright, high-contrast image of the labeled structures against a dark background.

**291.** (B) Dichroic mirror and emission filter
To produce a clean fluorescent image, the microscope must separate the bright excitation light from the much weaker emitted light. This is achieved using a dichroic mirror, which reflects short-wavelength excitation light toward the sample but allows long-wavelength emitted

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light to pass through. An additional emission filter then blocks any remaining excitation light, ensuring that only the specific fluorescent signal from the target molecule reaches the eye or the digital camera.

**292.** (B) Pinhole aperture to eliminate out-oF-focus light from planes above and below the focal plane
A major limitation of standard fluorescence microscopy is "blur" caused by light from parts of the specimen outside the focal plane. Confocal microscopy overcomes this by using a laser to scan the sample and a small pinhole aperture placed in front of the detector. The pinhole physically blocks any light that is not coming from the precise focal point, resulting in much sharper images and allowing for the creation of clear three-dimensional reconstructions.

**293.** (A) The wavelength of an electron beam is thousands of times shorter than the wavelength of visible light.
The resolution of any microscope is limited by the wavelength of the radiation it uses. Visible light has a wavelength of hundreds of nanometers, which limits its resolution to about 200 nm. In contrast, high-speed electrons have extremely short wavelengths, often less than a nanometer. This allows transmission electron microscopes to resolve details at the atomic level, revealing the fine ultrastructure of organelles and even individual protein complexes.

**294.** (B) Scatter electrons to create contrast in the final image.
Biological molecules are composed primarily of light elements (C, H, O, N) that do not interact strongly with electrons. To see these structures in a TEM, the sample must be "stained" with heavy metal salts. These heavy atoms are electron-dense and scatter the electron beam as it passes through the thin section. This creates dark areas in the final image where the metals have bound, providing the contrast necessary to visualize the cell's internal components.

**295.** (C) Generating highly detailed 3D images of the exterior surface topology of a specimen
Scanning Electron Microscopy (SEM) is the preferred tool for studying the surface of a sample. Instead of passing through a thin section, a focused beam of electrons scans across the surface of the specimen. This triggers the emission of secondary electrons, which are captured by a detector to create an image. SEM provides a great depth of field, resulting in striking, three-dimensional-looking images of the external structure of cells, tissues, or microorganisms.

**296.** (D) Sputter-coating the exterior surface of the specimen with a thin layer of conductive metal, such as gold
To prevent the buildup of static electrical charge during imaging in an SEM, the surface of the specimen must be made conductive. This is achieved through "sputter-coating," where a very thin layer of a heavy metal, such as gold or platinum, is deposited onto the sample. This conductive coating allows the electrons from the beam to flow away from the surface, ensuring a stable and high-quality image of the specimen's exterior topology.

**297.** (C) 200 nm
Abbe's diffraction limit describes the fundamental physical boundary for the resolution of a light microscope. It states that the smallest distance between two points that can be distinguished is roughly equal to half the wavelength of the light being used. For visible light, this limit is approximately 200 nanometers. Any structure smaller than this, such as a ribosome or a small virus, will appear as a single blurred spot in a standard light microscope.

**298.** (C) The jellyfish Aequorea victoria
Green Fluorescent Protein (GFP) was originally discovered in the jellyfish Aequorea victoria, where it functions to convert blue light from another protein into green light. Scientists have since adapted the gene for use in modern cell biology. By fusing the GFP gene to a protein of interest, researchers can create "fluorescent tags" that allow them to track the movement and behavior of specific proteins inside living cells in real time using a fluorescence microscope.

**299.** (A) Pseudo-3D, shadow-cast appearance
Differential Interference Contrast (DIC) microscopy, also known as Nomarski interference contrast, utilizes polarized light and specialized prisms to enhance contrast. Like phase-contrast, it detects differences in the optical path length within a specimen. However, the unique way the light is recombined in a DIC microscope creates an image where structures appear to have shadows, giving the final result a distinct three-

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dimensional, relieF-like quality that is excellent for visualizing surface details.

**300.** (B) Immunofluorescence
Immunofluorescence is a powerful microscopy technique that utilizes the specificity of antibodies to target particular proteins. Primary antibodies bind to the protein of interest, and secondary antibodies conjugated to fluorophores bind to the primary ones. When illuminated at a specific wavelength, these fluorophores emit light, allowing researchers to visualize the precise location and relative abundance of proteins within cells or tissue sections with high sensitivity and specificity.

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# CHAPTER 02
# Molecular Biology

2023

**1.** Which foundational principle was demonstrated by Griffith's in vivo experiments using Streptococcus pneumoniae?
(A) Transforming principle demonstration
(B) Evidence that DNA serves as the unique carrier of all bacterial genetic information
(C) Verification that polysaccharide capsules provide the structural blueprint for heredity
(D) Proof that heat-killed virulent strains undergo rapid and spontaneous mutation events

2024

**2.** Avery, MacLeod, and McCarty extended Griffith's work by utilizing which class of enzymes to identify the transforming principle?
(A) Lipases, amylases, and nucleases
(B) Endonucleases, exonucleases, and ligases
(C) Proteases, RNases, and DNases
(D) Topoisomerases, helicases, and polymerases

2008

**3.** In the Hershey-Chase experiment, which radioactive isotopes were used to specifically label viral DNA and proteins, respectively?
(A) Phosphorus-32 and Sulfur-35
(B) Phosphorus-32 and Carbon-14
(C) Sulfur-35 and Phosphorus-32
(D) Nitrogen-15 and Sulfur-35

2004

**4.** What was the critical experimental evidence from Fraenkel-Conrat's research on the Tobacco Mosaic Virus (TMV)?
(A) Pathogenic TMV isolates utilize double-stranded DNA as the primary genome
(B) Viral protein capsids determine the host specificity of the pathogen
(C) Plant viruses are capable of autonomous replication without host cells
(D) RNA is the genetic material in TMV

2016

**5.** The transformation observed in Griffith's experiment required the uptake of DNA. What natural bacterial state facilitates this process?
(A) Lysogeny
(B) Competence
(C) Conjugation
(D) Transduction

2016

**6.** Why did Avery, MacLeod, and McCarty's conclusion initially face resistance from the scientific community?
(A) The experimental methodology lacked a rigorous negative control group
(B) The specific bacterial strains utilized were significantly mutated
(C) DNA was perceived as chemically too simple
(D) Transformation by proteins was observed in concurrent research studies

2025

**7.** The Hershey-Chase blender experiment relied on which specific physical process to separate viral coats from infected bacterial cells?
(A) Equilibrium density gradient centrifugation using cesium chloride salts
(B) Localized high-temperature denaturation of the outer viral capsid
(C) Targeted enzymatic digestion of the bacterial peptidoglycan cell wall
(D) Mechanical shearing by a laboratory blender

2005

**8.** In Fraenkel-Conrat's reconstitution experiment, hybrid TMV particles were assembled from RNA of type A and protein of type B. The resulting progeny had which composition?
(A) Type A RNA and Type A protein
(B) Type A RNA and Type B protein
(C) Type B RNA and Type B protein
(D) Type B RNA and Type A protein

2010

**9.** Which biochemical characteristic makes the phosphorus isotope ideal for tracking DNA in the Hershey-Chase experiment?
(A) Phosphorus is absent in all known biological proteins
(B) The phosphate backbone is exclusive to nucleic acids among macromolecules
(C) Phosphorus emits a stronger beta particle

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than sulfur
(D) It rapidly integrates into the bacterial cell wall

2021
**10.** Which statement INCORRECTLY describes the experimental design of Avery, MacLeod, and McCarty?
(A) Smooth strain cell extracts were fractionated into specific macromolecular classes
(B) RNase treatment completely abolished the transforming activity
(C) DNase treatment destroyed the ability to convert R cells to S cells
(D) Protease treatment had no effect on the transformation efficiency

2015
**11.** What is the fundamental distinction between the transformation demonstrated by Griffith and the transduction seen in bacteriophages?
(A) Transformation involves naked DNA, whereas transduction requires a viral vector
(B) Transformation requires cell-to-cell contact, unlike transduction
(C) Transduction only transfers extrachromosomal plasmids
(D) Transformation is restricted to eukaryotic host cells

2014
**12.** Match the items in List I with the items in List II.
List I: (Researchers)
P. Griffith
Q. Avery, MacLeod, McCarty
R. Hershey and Chase
S. Fraenkel-Conrat
List II: (Contribution)
1. RNA as genetic material in TMV
2. Viral DNA enters host cells while protein does not
3. Biochemical identification of the transforming principle
4. Discovery of bacterial transformation
(A) P-3, Q-4, R-1, S-2
(B) P-4, Q-2, R-3, S-1
(C) P-4, Q-3, R-2, S-1
(D) P-1, Q-3, R-4, S-2

2019
**13.** Which nitrogenous base is a purine derivative containing an amino group at the C6 position?
(A) Guanine
(B) Cytosine
(C) Thymine
(D) Adenine

2001
**14.** What type of covalent bond connects the nitrogenous base to the pentose sugar in a nucleoside?
(A) Phosphodiester bond
(B) Hydrogen bond
(C) N-glycosidic bond
(D) Anhydride bond

2025
**15.** In a standard nucleotide, to which carbon atom of the ribose or deoxyribose sugar is the primary phosphate group attached?
(A) 1' carbon
(B) 2' carbon
(C) 3' carbon
(D) 5' carbon

2025
**16.** Which chemical modification distinguishes thymine from uracil?
(A) An amino group at the C4 position
(B) A hydroxyl group at the C2 position
(C) A carbonyl group at the C6 position
(D) A methyl group at the C5 position

2003
**17.** The phosphodiester linkages in a DNA polynucleotide chain connect which two carbon atoms of adjacent sugar residues?
(A) 3' and 5'
(B) 5' and 5'
(C) 2' and 5'
(D) 1' and 3'

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2011
**18.** In the following question, a statement of Assertion (A) is followed by a statement of Reason (R). Choose the correct option.
**Assertion (A):** The RNA molecule is generally less chemically stable than the DNA molecule under alkaline conditions.
**Reason (R):** The presence of a 2'-hydroxyl group on the ribose sugar makes RNA susceptible to base-catalyzed hydrolysis.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2012
**19.** What is the correct systematic name for the nucleoside containing guanine and ribose?
(A) Guanosine
(B) Deoxyguanosine
(C) Guanylate
(D) Guanine monophosphate

2021
**20.** Which of the following best defines a pyrimidine base?
(A) A double-ringed heterocyclic molecule containing carbon and nitrogen
(B) A specialized nucleoside unit lacking a terminal phosphate group
(C) A nitrogenous base that pairs exclusively with uracil in DNA
(D) A single-ring heterocyclic structure

2016
**21.** Which molecule serves as the structural backbone of both DNA and RNA?
(A) Nitrogenous bases linked by hydrogen bonds
(B) Alternating pentose sugars and phosphate groups
(C) Polypeptide chains bound to nucleotides
(D) Repeating purine-pyrimidine dimers

2023
**22.** What is the fundamental difference between a nucleoside and a nucleotide?
(A) A nucleotide lacks a nitrogenous base
(B) A nucleotide contains ribose, while a nucleoside contains deoxyribose
(C) A nucleoside lacks a phosphate group
(D) A nucleoside incorporates only pyrimidines

2024
**23.** During polynucleotide synthesis, the addition of a new nucleotide releases which byproduct?
(A) Water
(B) Ammonia
(C) Adenine triphosphate
(D) Pyrophosphate

2005
**24.** Consider the following statements regarding nucleotide structures:
I. Cytosine contains a carbonyl group at C2 and an amino group at C4.
II. Adenine forms three hydrogen bonds with thymine.
III. Purines are synthesized directly on the ribose sugar.
Which of the above statement(s) is/are correct?
(A) I and II
(B) I and III
(C) II and III
(D) I, II and III

2022
**25.** Which DNA conformation represents the standard Watson-Crick double helix under physiological conditions?
(A) A-DNA
(B) B-DNA
(C) Z-DNA
(D) C-DNA

2006
**26.** What happens to the ultraviolet absorbance of DNA when it undergoes complete denaturation?
(A) It decreases due to the hypochromic effect
(B) It increases due to the hyperchromic effect
(C) It remains constant regardless of the structural state
(D) It shifts to a higher wavelength maximum

2019
**27.** The melting temperature (Tm) of a DNA molecule is directly proportional to its content of which base pair?
(A) Guanine-Cytosine
(B) Adenine-Thymine
(C) Adenine-Uracil
(D) Cytosine-Thymine

2016
**28.** Which structural feature explicitly defines Z-DNA in contrast to B-DNA?
(A) Right-handed helical rotation
(B) Complete absence of a minor groove
(C) Left-handed helical rotation
(D) Parallel orientation of the two strands

2006
**29.** A-DNA typically forms under which environmental condition?
(A) High aqueous hydration
(B) Extremely high temperature
(C) Low salt concentration
(D) Dehydrating conditions or low humidity

2016
**30.** Choose the correct sequence of DNA conformational changes when shifting from a high hydration state to an extremely dehydrated state containing high salt and alternating GC sequences.
(A) A-DNA → B-DNA → Z-DNA
(B) Z-DNA → A-DNA → B-DNA
(C) B-DNA → A-DNA → Z-DNA

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(D) B-DNA → Z-DNA → A-DNA

2017
**31.** Which thermodynamic force is the primary driver of DNA base stacking interactions?
(A) Covalent bonding between adjacent bases
(B) Hydrophobic interactions and van der Waals forces
(C) Electrostatic repulsion of the phosphate groups
(D) Hydrogen bonding between complementary strands

2013
**32.** In the B-DNA model, how many base pairs are typically present per complete helical turn?
(A) 9.5
(B) 10.5
(C) 11.0
(D) 12.0

2018
**33.** What is the approximate width (diameter) of the standard B-DNA double helix?
(A) 10 Angstroms
(B) 20 Angstroms
(C) 30 Angstroms
(D) 34 Angstroms

2009
**34.** Which phenomenon facilitates the spontaneous renaturation of a denatured DNA double helix upon slow cooling?
(A) Homologous recombination
(B) Ligase-mediated covalent bonding
(C) Base pairing and stacking re-establishment
(D) Supercoiling-induced structural stress

2015
**35.** The major and minor grooves of DNA arise structurally due to which geometric property?
(A) Asymmetrical attachment of base pairs to the backbone
(B) The specific antiparallel orientation of the phosphodiester backbones
(C) The repetitive alternating sequences of purines and pyrimidines
(D) The presence of a varying number of stabilizing hydrogen bonds

2016
**36.** Match the items in List I with the items in List II.
List I: (DNA Conformation)
P. A-DNA
Q. B-DNA
R. Z-DNA
S. Denatured DNA
List II: (Characteristic)
1. Left-handed helix with zigzag backbone
2. 11 base pairs per turn, right-handed
3. High UV absorbance at 260 nm
4. 10.5 base pairs per turn, biological standard
(A) P-4, Q-2, R-1, S-3
(B) P-1, Q-4, R-2, S-3
(C) P-2, Q-3, R-1, S-4
(D) P-2, Q-4, R-1, S-3

2017
**37.** Which type of cellular RNA is the most abundant in a functionally active, rapidly growing bacterial cell?
(A) mRNA
(B) rRNA
(C) tRNA
(D) snRNA

2017
**38.** The characteristic cloverleaf secondary structure is exclusively associated with which RNA molecule?
(A) Messenger RNA
(B) Ribosomal RNA
(C) Transfer RNA
(D) Small nuclear RNA

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2013
**39.** In eukaryotes, which class of RNA is primarily involved in the splicing of pre-mRNA transcripts?
(A) MicroRNAs
(B) Small nucleolar RNAs (snoRNAs)
(C) Ribosomal RNAs
(D) Small nuclear RNAs (snRNAs)

2022
**40.** Select the correct order of RNA types from shortest average halF-life to longest halF-life in a typical prokaryotic cell.
(A) mRNA → tRNA → rRNA
(B) rRNA → tRNA → mRNA
(C) tRNA → mRNA → rRNA
(D) mRNA → rRNA → tRNA

2010
**41.** Which unique structural feature is present at the 3' end of all mature transfer RNA (tRNA) molecules?
(A) A poly(A) tail
(B) A CCA trinucleotide sequence
(C) An inverted guanosine cap
(D) A modified pseudouridine residue

2009

**42.** In the following question, a statement of Assertion (A) is followed by a statement of Reason (R). Choose the correct option.

**Assertion (A):** Eukaryotic mRNA undergoes extensive processing before translation, whereas prokaryotic mRNA does not.

**Reason (R):** Transcription and translation are spatially and temporally uncoupled in eukaryotic cells.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2023

**43.** Regulatory small RNAs (sRNAs) in bacteria primarily control gene expression through which mechanism?

(A) Base-pairing with target mRNAs to alter their translation or stability
(B) Inducing direct DNA methylation at promoter regions
(C) Binding directly to the core RNA polymerase enzyme
(D) Acting as competitive inhibitors for ribosomal binding sites

2018

**44.** What is the functional role of the anticodon loop in a tRNA molecule?

(A) It covalently binds to the appropriate amino acid
(B) It interacts directly with the 50S ribosomal subunit
(C) It pairs with the complementary codon sequence on the mRNA
(D) It stabilizes the tertiary L-shaped structure of the tRNA

2002

**45.** Which specialized RNA functions to guide chemical modifications, such as 2'-O-methylation, on ribosomal RNAs in eukaryotes?

(A) snRNA
(B) siRNA
(C) lncRNA
(D) snoRNA

2022

**46.** The structural complexity of ribosomal RNA (rRNA) allows it to function biologically as which type of molecule?

(A) An informational template only
(B) A membrane transport carrier
(C) A structural scaffold and a ribozyme
(D) A generalized transcriptional repressor

2014

**47.** Which statement INCORRECTLY characterizes microRNAs (miRNAs)?

(A) They are synthesized via transcription from specific endogenous genomic loci
(B) They originate as long precursors known as primary transcripts (pri-miRNAs)
(C) Their major biological function involves post-transcriptional gene silencing
(D) Forming perfect matches with target mRNAs in animals

2001

**48.** Consider the following statements regarding RNA structure and folding:

I. tRNA assumes an L-shaped tertiary conformation.
II. RNA sequences cannot form double-helical regions.
III. Non-canonical base pairs (like G-U) are frequently found in RNA secondary structures.
IV. The D-loop in tRNA contains dihydrouridine.

Which of the above statement(s) is/are INCORRECT?

(A) I and III
(B) II only
(C) II and IV
(D) III and IV

2004

**49.** The primary structure of a protein is defined exclusively by which type of interaction?

(A) Hydrogen bonding between backbone atoms
(B) Peptide bonds connecting consecutive amino acid residues
(C) Hydrophobic interactions among side chains
(D) Disulfide bridges between distinct polypeptide chains

2018

**50.** Consider the following statements concerning the properties of the peptide bond:

I. It possesses partial double-bond character.
II. It allows completely free rotation around the C-N axis.
III. It typically adopts a trans configuration.

Which of the above statement(s) is/are correct?

(A) I and II
(B) II and III
(C) I and III

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(D) I, II and III

2002

**51.** The Ramachandran plot graphically visualizes the allowable values for which specific molecular parameters?
(A) Phi ($\phi$) and Psi ($\psi$) dihedral angles of the backbone
(B) Precise distances between adjacent alpha-carbon atoms in the chain
(C) Total number of hydrogen bond lengths within a standard alpha-helix
(D) The specific side-chain rotational orientations known as Chi angles

2017

**52.** Which secondary structure motif is characterized by a right-handed coil with 3.6 amino acid residues per turn?
(A) Beta-pleated sheet
(B) Collagen triple helix
(C) Beta-turn
(D) Alpha-helix

2011

**53.** What structural feature uniquely defines a beta-turn in a polypeptide chain?
(A) A chain reversal stabilized by an $i$ to $i+3$ hydrogen bond
(B) A rigid, fully extended polypeptide structure lacking any hydrogen bonding
(C) Intermolecular disulfide bridges that stabilize a series of parallel sheets
(D) A motif characterized by the absolute absence of proline or glycine residues

2003

**54.** Select the correct sequential order of structural complexity in protein organization.
(A) Motif → Domain → Tertiary → Quaternary
(B) Motif → Tertiary → Domain → Quaternary
(C) Domain → Motif → Quaternary → Tertiary
(D) Domain → Quaternary → Motif → Tertiary

2017

**55.** What non-covalent force is the principal driving factor in stabilizing the overall tertiary folding of globular proteins in an aqueous environment?
(A) Formation of ionic salt bridges across the external protein surface
(B) Hydrophobic effect burying nonpolar side chains in the interior
(C) Hydrogen bonding interactions occurring between all polar side chains
(D) Establishment of covalent disulfide cross-links between distant residues

NODIA

2002

**56.** Quaternary structure refers explicitly to which level of protein organization?
(A) The spatial arrangement of multiple independent polypeptide subunits
(B) The three-dimensional fold of a single continuous polypeptide chain
(C) The local periodic arrangement of the protein backbone
(D) The interaction between a protein and a non-protein prosthetic group

2004

**57.** Which amino acid is heavily restricted in the Ramachandran plot due to its cyclic side chain that limits rotational flexibility?
(A) Glycine
(B) Alanine
(C) Proline
(D) Tryptophan

2013

**58.** Conversely, which amino acid has the broadest allowable range of conformations on the Ramachandran plot due to its lack of a bulky side chain?
(A) Glycine
(B) Leucine
(C) Phenylalanine
(D) Valine

2014

**59.** The denaturant urea disrupts protein tertiary structure primarily by interfering with which type of molecular interaction?
(A) Peptide bonds
(B) Disulfide bonds
(C) Hydrogen bonding and hydrophobic interactions
(D) Primary amino acid sequence

2017

**60.** Match the items in List I with the items in List II.
List I: (Protein Structure Level)
P. Primary
Q. Secondary
R. Tertiary
S. Quaternary
List II: (Primary Stabilizing Force)
1. Subunit interactions (non-covalent)
2. Peptide covalent bonds
3. Backbone hydrogen bonds
4. Side-chain interactions (hydrophobic, ionic, etc.)
(A) P-2, Q-3, R-4, S-1

(B) P-3, Q-2, R-1, S-4
(C) P-2, Q-4, R-3, S-1
(D) P-1, Q-2, R-3, S-4

2010

**61.** The prokaryotic nucleoid differs fundamentally from a eukaryotic nucleus in which structural aspect?
(A) The nucleoid contains strictly linear DNA molecules
(B) The nucleoid is not enclosed by a distinct lipid bilayer membrane
(C) The nucleoid completely lacks associated structural proteins
(D) The nucleoid undergoes independent mitotic division

2017

**62.** Most bacterial chromosomes are maintained in a specific topological state. What is this state?
(A) Positively supercoiled circular DNA
(B) Relaxed linear DNA
(C) Highly condensed linear chromosomes
(D) Negatively supercoiled circular DNA

2018

**63.** Which bacterial enzyme is specifically responsible for introducing negative supercoils into DNA utilizing ATP hydrolysis?
(A) DNA Helicase
(B) Topoisomerase I
(C) DNA Ligase
(D) DNA Gyrase (Topoisomerase II)

2002

**64.** What happens to the linking number (Lk) of a closed circular DNA molecule when DNA gyrase acts upon it?
(A) It increases by increments of 1
(B) It remains constant while writhe changes
(C) It decreases by increments of 2
(D) It fluctuates randomly depending on ATP concentration

2025

**65.** The architectural organization of the bacterial nucleoid involves independent topological units known as what?
(A) Nucleosomes
(B) Supercoiled macrodomains or loops
(C) Solenoid fibers
(D) Centromeric regions

2021

**66.** In the following question, a statement of Assertion (A) is followed by a statement of Reason (R). Choose the correct option.
**Assertion (A):** Topoisomerase I relaxes negative supercoils in bacterial DNA without the requirement of ATP.
**Reason (R):** Topoisomerase I breaks only one strand of the DNA double helix, allowing the unbroken strand to pass through the break before resealing.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2014

**67.** Which family of small basic proteins functions analogously to eukaryotic histones in bacteria to compact the nucleoid?
(A) High Mobility Group (HMG) proteins
(B) Scaffold attachment factors
(C) Protamines
(D) Nucleoid-associated proteins (NAPs) such as HU and IHF

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2001

**68.** Quinolone antibiotics, such as ciprofloxacin, exert their bactericidal effect by targeting which crucial enzyme?
(A) DNA Gyrase
(B) RNA Polymerase
(C) DNA Polymerase III
(D) Ribosomal peptidyl transferase

2022

**69.** When a bacterial DNA molecule is isolated, a fraction of it is found to be positively supercoiled. Which enzyme is most likely responsible for resolving this state ahead of the replication fork?
(A) Topoisomerase I
(B) DNA Gyrase
(C) Topoisomerase IV
(D) Single-strand binding protein (SSB)

2013

**70.** How does the enzyme Topoisomerase IV primarily function during the terminal stages of bacterial DNA replication?
(A) Initiation of chromosomal replication at the unique oriC locus
(B) Decatenation of intertwined daughter chromosomes
(C) Synthesis of small RNA primers required for

Okazaki fragments
(D) Introduction of positive supercoiling to condense the nucleoid

2019
**71.** If the specific linking difference ($\sigma$) of a bacterial plasmid is approximately -0.06, what does this value indicate?
(A) DNA is underwound, resulting in negative supercoiling
(B) The DNA molecule is positively supercoiled by exactly 6 percent
(C) The circular DNA molecule exists in a completely relaxed state
(D) The DNA backbone has suffered catastrophic double-strand breaks

2022
**72.** Consider the following statements regarding DNA supercoiling:
I. Twist refers to the coiling of the double helix axis over itself.
II. Writhe refers to the number of helical turns of one strand about the other.
III. $Lk = Tw + Wr$ for any closed circular DNA.
Which of the above statement(s) is/are INCORRECT?
(A) I and II
(B) I and III
(C) II and III
(D) I, II and III

2023
**73.** The fundamental repetitive structural unit of eukaryotic chromatin is the nucleosome. What is its basic composition?
(A) 147 base pairs of DNA wrapped around a simple hexamer of histones
(B) 200 base pairs of DNA bound to non-histone chromosomal scaffold proteins
(C) A continuous DNA supercoil interspersed with linker $H_1$ protein molecules
(D) 147 bp of DNA wrapped around an octamer of core histones

2016
**74.** Which specific histone proteins constitute the core octamer of the nucleosome?
(A) One copy each of $H_1$, $H_2A$, $H_2B$, and $H_3$
(B) Two copies each of $H_1$, $H_2$, $H_3$, and $H_4$
(C) Two copies each of $H_2A$, $H_2B$, $H_3$, and $H_4$
(D) Four copies each of $H_3$ and $H_4$

2011
**75.** What is the primary functional role of the linker histone $H_1$ in chromatin organization?
(A) Formation of the central nucleosomal core during the initial folding
(B) Compaction of nucleosomes into the 30 nm fiber
(C) Active transcription of all available euchromatic genomic regions
(D) Enzymatic degradation of naked DNA segments located between nucleosomes

2004
**76.** In the solenoid model of the 30 nm chromatin fiber, approximately how many nucleosomes constitute a single helical turn?
(A) 2
(B) 4
(C) 6
(D) 10

2001
**77.** Which biochemical property allows histone proteins to bind tightly to the DNA phosphate backbone?
(A) An abundance of basic amino acids like lysine and arginine
(B) An abundance of acidic amino acids like aspartate and glutamate
(C) The presence of heavy metal cofactors
(D) A high proportion of aromatic residues

2005
**78.** Determine the correct hierarchical arrangement of eukaryotic chromatin compaction from least condensed to most condensed.
(A) Naked DNA → 30 nm fiber → Nucleosome → Metaphase chromosome
(B) Naked DNA → Nucleosome → 30 nm fiber → Radial loop domains → Metaphase chromosome
(C) Nucleosome → Naked DNA → Radial loop domains → 30 nm fiber → Metaphase chromosome
(D) Naked DNA → Radial loop domains → Nucleosome → 30 nm fiber → Metaphase chromosome

2005
**79.** Heterochromatin is generally characterized by which of the following transcriptional states?
(A) Highly active, hyperacetylated, and unmethylated
(B) Transcriptionally active but localized to the nuclear periphery
(C) Constitutively active across all developmental stages
(D) Transcriptionally inert, heavily methylated,

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and hypoacetylated

2003

**80.** The acetylation of core histone tails generally results in which physiological outcome?
(A) Tighter binding of DNA to histones, resulting in suppressed transcription
(B) Chromatin relaxation, facilitating transcriptional activation
(C) Immediate and complete degradation of the central nucleosome core particle
(D) Rapid conversion of euchromatin into permanent constitutive heterochromatin

2021

**81.** Constitutive heterochromatin is permanently condensed and typically located at which specific chromosomal regions?
(A) Telomeres and centromeres
(B) Gene-rich promoters and enhancers
(C) Actively transcribed ribosomal DNA arrays
(D) Coding sequences of housekeeping genes

2023

**82.** Which specific domain on core histone proteins is subjected to extensive post-translational modifications (PTMs)?
(A) The globular C-terminal domain
(B) The structured histone-fold motif
(C) The unstructured N-terminal tail
(D) The central hydrophobic core

2010

**83.** Facultative heterochromatin differs from constitutive heterochromatin in that it:
(A) Remains in a permanently condensed state throughout all cell lineages
(B) Consists exclusively of highly repetitive satellite DNA sequences
(C) Exhibits a complete absence of histone $H_3$ within the nucleosome
(D) Contains genes silenced in specific cell types but activatable in others

2021

**84.** Match the items in List I with the items in List II.
List I: (Chromatin State/Feature)
P. Euchromatin
Q. Constitutive heterochromatin
R. Nucleosome
S. Scaffold proteins
List II: (Characteristic)
1. Structural maintenance of high-order chromosome loops
2. Centromeric and telomeric regions
3. Transcriptionally active and less condensed
4. DNA wrapped around a histone octamer
(A) P-3, Q-2, R-4, S-1
(B) P-2, Q-3, R-1, S-4
(C) P-3, Q-1, R-4, S-2
(D) P-1, Q-4, R-3, S-2

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2019

**85.** The origin of replication in E. coli, oriC, is primarily characterized by the presence of which specific structural motifs?
(A) Clusters of highly GC-content repetitive sequences and motifs
(B) Consensus TATA box sequences utilized for transcriptional control
(C) Termination (Ter) sites which stop the progress of replication
(D) DnaA binding boxes and AT-rich 13-mer regions

2006

**86.** Which protein serves as the primary initiator in prokaryotic DNA replication by recognizing and binding to oriC?
(A) DnaB
(B) DnaC
(C) DnaG
(D) DnaA

2004

**87.** During the initiation of DNA replication in E. coli, what is the specific role of the DnaC protein?
(A) It synthesizes the initial RNA primer
(B) It stabilizes single-stranded DNA
(C) It resolves positive supercoils ahead of the fork
(D) It acts as a helicase loader, delivering DnaB to the replication fork

2023

**88.** Okazaki fragments are synthesized on which strand during bidirectional DNA replication?
(A) The leading strand
(B) The lagging strand
(C) Both leading and lagging strands
(D) The continuous strand

2002

**89.** Which enzyme is responsible for synthesizing the short RNA primers necessary to initiate Okazaki fragment synthesis in E. coli?
(A) DNA Polymerase I
(B) RNase H
(C) DNA Ligase
(D) Primase (DnaG)

2013

**90.** In the following question, a statement of Assertion (A) is followed by a statement of Reason (R). Choose the correct option.

**Assertion (A):** Synthesis on the lagging strand proceeds in a discontinuous manner.

**Reason (R):** DNA polymerases can only synthesize DNA in the 5' to 3' direction, dictating that one strand must be synthesized in short segments as the replication fork unwinds.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2002

**91.** In E. coli, which specific enzyme primarily removes the RNA primers and replaces them with DNA?

(A) DNA Polymerase I
(B) DNA Polymerase II
(C) DNA Polymerase III
(D) DNA Ligase

2018

**92.** The replication of the E. coli chromosome terminates when the replication forks meet at specific sequences known as Ter sites. Which protein binds to these sites to halt helicase activity?

(A) FtsZ protein
(B) RecA protein
(C) LexA protein
(D) Tus protein

2015

**93.** What is the fundamental biochemical function of DNA Ligase during prokaryotic replication?

(A) To initiate the unwinding of the double helix
(B) To synthesize the final Okazaki fragment
(C) To remove incorrect nucleotides via exonuclease activity
(D) To catalyze the formation of a phosphodiester bond between adjacent DNA fragments

2019

**94.** Single-strand binding proteins (SSBs) are crucial at the replication fork because they:

(A) Unwind the DNA double helix
(B) Prevent single-stranded DNA from reannealing or forming secondary structures
(C) Provide a free 3'-OH group for polymerase extension
(D) Actively degrade old RNA primers

2002

**95.** The replisome complex ensures coordinated synthesis of both leading and lagging strands. This is mechanistically explained by which model?

(A) The solenoid model
(B) The trombone model
(C) The sliding clamp model
(D) The end-replication model

2025

**96.** Consider the following statements concerning prokaryotic DNA replication:

I. Replication is semi-conservative and bidirectional.
II. DnaB acts as a 5' to 3' helicase.
III. The lagging strand template is looped to orient the polymerase in the correct direction.
IV. Termination strictly requires the action of topoisomerase II.

Which of the above statement(s) is/are correct?

(A) I, II and III
(B) I, III and IV
(C) II, III and IV
(D) I, II, III and IV

2008

**97.** How does the initiation of DNA replication in eukaryotes fundamentally differ from that in prokaryotes?

(A) Eukaryotes possess multiple origins per chromosome
(B) Eukaryotes utilize a single, massive origin of replication for each chromosome
(C) Eukaryotic replication proceeds only in the 3' to 5' direction on both strands
(D) Eukaryotic replication initiation does not require a short RNA primer sequence

2023

**98.** In the eukaryotic cell cycle, the assembly of the pre-replication complex (pre-RC) occurs during which specific phase?

(A) $G_1$ phase
(B) S phase
(C) $G_2$ phase
(D) M phase

2012

**99.** Which multiprotein complex binds to eukaryotic origins of replication to initiate the formation of the pre-RC?

(A) Origin Recognition Complex (ORC)
(B) Minichromosome Maintenance (MCM) complex

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(C) Proliferating Cell Nuclear Antigen (PCNA)
(D) Replication Protein A (RPA)

2001

**100.** Cell cycle regulation strictly limits eukaryotic origin firing to once per cycle. Which factors are collectively degraded or exported to prevent re-initiation during the S phase?
(A) ORC subunits
(B) DNA polymerases
(C) Licensing factors such as Cdc6 and Cdt1
(D) Telomerases

2009

**101.** What is the primary function of the MCM helicase complex in eukaryotes?
(A) Efficiently loading PCNA sliding clamps onto the parental DNA strands
(B) Unwinding the DNA double helix ahead of the fork
(C) Directly synthesizing the leading strand in a continuous fashion
(D) Enzymatically removing RNA primers from the nascent lagging strand

2002

**102.** Select the correct sequence of events during the initiation of eukaryotic DNA replication.
(A) ORC binding → Cdc6/Cdt1 recruitment → MCM loading → Kinase activation (CDK/DDK)
(B) MCM loading → ORC binding → Cdc6/Cdt1 recruitment → Kinase activation
(C) Kinase activation → ORC binding → MCM loading → Cdc6/Cdt1 recruitment
(D) ORC binding → Kinase activation → MCM loading → Cdc6/Cdt1 recruitment

2021

**103.** The linear nature of eukaryotic chromosomes presents a unique "end-replication problem." This specifically refers to the inability to completely replicate which region?
(A) The 5' end of the leading strand
(B) The 3' end of the lagging strand template
(C) The centromeric heterochromatin
(D) The core origin sequences

2015

**104.** Telomerase solves the end-replication problem by utilizing which distinctive mechanism?
(A) Operates as a standard DNA-dependent DNA polymerase during extension
(B) Acts as a reverse transcriptase with its own RNA template
(C) Synthesizes DNA in a linear fashion starting from a free 5' end
(D) Catalyzes the ligation of circular DNA intermediates to form the ends

2005

**105.** Telomeres are typically composed of characteristic tandem repeat sequences. In human cells, what is this core repeat sequence?
(A) 5'-TTAGGG-3'
(B) 5'-CCCTAA-3'
(C) 5'-GGTTAA-3'
(D) 5'-AATCCC-3'

2014

**106.** Which structural conformation protects the single-stranded overhang at the extreme ends of mammalian telomeres from being recognized as DNA damage?
(A) D-loop
(B) Holliday junction
(C) T-loop
(D) Cruciform structure

2010

**107.** Telomerase activity is generally repressed in normal human somatic cells. What is the primary physiological consequence of this repression?
(A) Unregulated and continuous cell division resulting in tissue overgrowth
(B) Progressive telomere shortening with division, leading to senescence
(C) Immediate oncogenic transformation of all somatic cell populations
(D) Accumulation of massive positive supercoiling within the telomeric DNA

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2008

**108.** Match the items in List I with the items in List II.
List I: (Eukaryotic Replication Component)
P. ORC
Q. MCM complex
R. PCNA
S. RPA
List II: (Prokaryotic Analog)
1. Single-strand binding protein (SSB)
2. Beta sliding clamp
3. DnaB helicase
4. DnaA protein
(A) P-4, Q-3, R-2, S-1
(B) P-3, Q-4, R-1, S-2
(C) P-4, Q-2, R-3, S-1
(D) P-2, Q-3, R-4, S-1

2009

**109.** Which catalytic capability is shared universally among all known DNA polymerases?
(A) Robust 3' to 5' exonuclease activity for proofreading the sequence
(B) Inherent 5' to 3' exonuclease activity for removing RNA primers
(C) Unique ability to initiate DNA synthesis de novo without a primer
(D) 5' to 3' polymerase activity requiring a primer

2004

**110.** In E. coli, which DNA polymerase is the principal enzyme responsible for bulk chromosomal replication?
(A) DNA Polymerase I
(B) DNA Polymerase II
(C) DNA Polymerase III
(D) DNA Polymerase IV

2014

**111.** What unique catalytic activity allows E. coli DNA Polymerase I to perform nick translation and RNA primer removal?
(A) 5' to 3' exonuclease activity
(B) 3' to 5' exonuclease activity
(C) Endonuclease activity
(D) Ribonuclease H activity exclusively

2014

**112.** The high fidelity of DNA Polymerase III is primarily attributed to which specific proofreading mechanism?
(A) Pre-synthetic error avoidance
(B) 5' to 3' exonuclease activity excising mismatched bases
(C) Post-replication mismatch repair only
(D) 3' to 5' exonuclease activity of the epsilon ($\varepsilon$) subunit

2023

**113.** In eukaryotes, which DNA polymerase contains intrinsic primase activity and synthesizes the initial RNA-DNA primer?
(A) DNA Polymerase delta ($\delta$)
(B) DNA Polymerase epsilon ($\varepsilon$)
(C) DNA Polymerase gamma ($\gamma$)
(D) DNA Polymerase alpha ($\alpha$)

2003

**114.** In the following question, a statement of Assertion (A) is followed by a statement of Reason (R). Choose the correct option.
**Assertion (A):** Eukaryotic DNA Polymerase epsilon ($\varepsilon$) is primarily responsible for synthesizing the leading strand.
**Reason (R):** DNA Polymerase epsilon is highly processive and possesses robust 3' to 5' proofreading activity.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2015

**115.** Which eukaryotic DNA polymerase is localized entirely to the mitochondria and is responsible for replicating the mitochondrial genome?
(A) DNA Polymerase beta ($\beta$)
(B) DNA Polymerase gamma ($\gamma$)
(C) DNA Polymerase delta ($\delta$)
(D) DNA Polymerase theta ($\theta$)

2018

**116.** Processivity in E. coli DNA Polymerase III is drastically enhanced by its association with which structural subunit?
(A) Alpha ($\alpha$) subunit
(B) Gamma ($\gamma$) complex loader
(C) Theta ($\theta$) subunit
(D) Beta ($\beta$) clamp

2008

**117.** What functional role is attributed to eukaryotic DNA Polymerase delta ($\delta$)?
(A) Primary synthesis of the lagging strand
(B) Mitochondrial replication exclusively
(C) Initial RNA primer synthesis
(D) Translesion synthesis across bulky adducts

2013

**118.** E. coli DNA Polymerase II is primarily induced during which specific cellular event?
(A) Standard exponential growth phase
(B) Initiation of plasmid replication
(C) The SOS response to DNA damage
(D) Termination of the replication fork

2009

**119.** A defining limitation of all DNA polymerases is their strict requirement for a free hydroxyl group to add the next nucleotide. To which carbon is this hydroxyl group attached?
(A) 3' Carbon
(B) 1' Carbon
(C) 2' Carbon
(D) 5' Carbon

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2018

**120.** Consider the following statements regarding DNA polymerases:

I. Prokaryotic DNA Pol I is a single polypeptide chain.

II. Eukaryotic DNA Pol alpha lacks 3' to 5' proofreading activity.

III. The Klenow fragment of Pol I retains 5' to 3' exonuclease activity.

IV. Processivity refers to the number of nucleotides added before the polymerase dissociates.

V. PCNA is the functional equivalent of the beta clamp in eukaryotes.

Which of the above statement(s) is/are correct?

(A) I, II, IV, V
(B) I, III, IV, V
(C) II, III, IV, V
(D) I, II, III, IV

2017

**121.** Exposure to high-energy ultraviolet (UV) radiation most commonly results in which specific type of DNA damage?

(A) Generation of widespread and lethal double-strand DNA breaks
(B) Formation of cyclobutane pyrimidine dimers
(C) Extensive depurination of guanine residues across the genome
(D) Direct deamination of cytosine to uracil within the DNA sequence

2010

**122.** Base Excision Repair (BER) is initiated by which specific class of enzymes that recognize and remove modified bases?

(A) Endonucleases
(B) DNA ligases
(C) Exinucleases
(D) DNA glycosylases

2005

**123.** The removal of a damaged base during BER creates an intermediate site in the DNA. What is this site called?

(A) A Holliday junction
(B) A bulky adduct
(C) An Okazaki fragment
(D) An AP (apurinic/apyrimidinic) site

2023

**124.** Nucleotide Excision Repair (NER) fundamentally differs from Base Excision Repair (BER) in that NER:

(A) Exclusively repairs the damage caused by specific alkylating agents
(B) Excises an oligonucleotide patch containing bulky lesions
(C) Targets and removes a single base without breaking the backbone
(D) Restricts its activity solely to the repair of double-strand breaks

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2024

**125.** In the E. coli mismatch repair (MMR) system, how do the repair enzymes distinguish the newly synthesized DNA strand from the parental template strand?

(A) The newly synthesized strand is highly methylated at GATC sequences
(B) The parental strand is transiently unmethylated
(C) The parental strand is methylated at the adenine of GATC sequences
(D) The newly synthesized strand is bound by single-strand binding proteins

2006

**126.** Which protein in the E. coli mismatch repair pathway is responsible for recognizing and binding to the mismatched base pair?

(A) MutH
(B) MutL
(C) MutS
(D) UvrA

2014

**127.** The bacterial SOS response is a global regulatory network activated by severe DNA damage. Which protein acts as the primary repressor of the SOS genes under normal conditions?

(A) RecA
(B) LexA
(C) UmuD
(D) Pol V

2005

**128.** How does the RecA protein facilitate the induction of the SOS response upon detecting single-stranded DNA?

(A) Functions as a transcription factor to activate SOS promoter regions
(B) Operates as a kinase to phosphorylate the LexA repressor protein
(C) Acts as a co-protease for LexA cleavage
(D) Directly synthesizes replacement DNA to fill in single-stranded gaps

2011

**129.** Translesion synthesis (TLS) during the SOS response utilizes specialized DNA polymerases

(like Pol IV and Pol V). What is the critical characteristic of these polymerases?
(A) Possess exceptionally high fidelity and robust proofreading capabilities
(B) Lack 3' to 5' proofreading and are highly error-prone
(C) Are restricted to the synthesis of initial RNA primer sequences only
(D) Function specifically to repair double-strand DNA breaks in the cell

2015
**130.** Double-strand breaks (DSBs) in eukaryotic DNA are exceptionally lethal. Which repair pathway rapidly rejoins the broken ends but is intrinsically error-prone due to the lack of a homologous template?
(A) Homologous recombination (HR)
(B) Nucleotide excision repair (NER)
(C) Mismatch repair (MMR)
(D) Non-homologous end joining (NHEJ)

2015
**131.** In the nucleotide excision repair (NER) pathway of E. coli, which protein complex acts as the specific excinuclease to cleave the damaged DNA strand on both sides of the lesion?
(A) UvrA/UvrB
(B) UvrB/UvrC
(C) UvrD
(D) DNA Polymerase I

2010
**132.** Consider the following statements concerning DNA repair mechanisms:
I. Deamination of adenine produces uracil.
II. NHEJ is the primary high-fidelity repair mechanism in bacteria.
III. Nucleotide excision repair (NER) is a major pathway for repairing ultraviolet-induced pyrimidine dimers in humans.
Which of the above statement(s) is/are INCORRECT?
(A) I and II
(B) I and III
(C) II and III
(D) I, II and III

2019
**133.** What is the fundamental composition of the E. coli RNA polymerase core enzyme?
(A) $\alpha_2\beta\beta'\omega$
(B) $\alpha_2\beta\beta'\sigma$
(C) $\alpha_2\beta\beta'\sigma\omega$
(D) $\alpha_2\beta_2\sigma$

2021
**134.** Which specific subunit must associate with the bacterial RNA polymerase core to form the functional holoenzyme capable of specific promoter recognition?
(A) Alpha ($\alpha$) subunit
(B) Beta ($\beta$) subunit
(C) Omega ($\omega$) subunit
(D) Sigma ($\sigma$) factor

2012
**135.** The canonical prokaryotic promoter contains two highly conserved consensus sequences. Where are these elements typically located relative to the transcription start site (+1)?
(A) -10 and -35 regions
(B) +10 and +35 regions
(C) -25 and -75 regions
(D) -50 and -100 regions

2018
**136.** The -10 consensus sequence in E. coli promoters, crucial for DNA unwinding during initiation, is also commonly known by what name?
(A) TATA box
(B) CAAT box
(C) GC box
(D) Pribnow box

2007
**137.** Which alternative sigma factor is specifically synthesized in E. coli to transcribe genes necessary for survival during extreme heat shock?
(A) Sigma 32 ($\sigma^{32}$)
(B) Sigma 70 ($\sigma^{70}$)
(C) Sigma 54 ($\sigma^{54}$)
(D) Sigma 28 ($\sigma^{28}$)

2008
**138.** The transition from a closed promoter complex to an open promoter complex during prokaryotic transcription initiation strictly requires which event?
(A) Hydrolysis of ATP by the RNA polymerase core enzyme itself
(B) Complete dissociation of the sigma factor from the holoenzyme
(C) Melting of DNA around the -10 element
(D) Binding of the specific transcription termination factor Rho to RNA

2002
**139.** Abortive initiation is a common feature of prokaryotic transcription. What does this process entail?

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(A) Premature termination of transcription at the terminal end of the gene
(B) Failure of RNA polymerase to recognize the -35 consensus sequence element
(C) Rapid enzymatic degradation of all full-length mRNA transcripts in the cell
(D) Synthesis and release of short RNAs (2-10 nt) during initiation

2012

**140.** Intrinsic (Rho-independent) termination of transcription in bacteria relies on which specific structural features in the nascent RNA transcript?
(A) GC-rich hairpin followed by a run of uracil residues
(B) A poly-A tail followed by a specialized protein binding sequence motif
(C) An extended sequence of alternating purine and pyrimidine nucleotides
(D) A terminal stop codon recognized by specific protein release factors

2009

**141.** How does the Rho protein facilitate transcription termination in Rho-dependent termination?
(A) Binds to the promoter region and blocks subsequent polymerase binding
(B) Cleaves the nascent RNA transcript internally at specific sequences
(C) Binds to the DNA template and acts as a physical roadblock to elongation
(D) Binds to a rut site and acts as an RNA-DNA helicase

2025

**142.** The transcription start site (+1) is most commonly which specific nucleotide in prokaryotic promoters?
(A) A purine (A or G)
(B) A pyrimidine (C or T)
(C) Exclusively Thymine
(D) Exclusively Cytosine

2006

**143.** During the elongation phase of prokaryotic transcription, which structural channel in the RNA polymerase channels the exiting nascent RNA transcript?
(A) The downstream DNA channel
(B) The NTP entry channel
(C) The primary catalytic cleft
(D) The RNA exit channel

2010

**144.** Which antibiotic exerts its bactericidal effect by binding specifically to the beta ($\beta$) subunit of bacterial RNA polymerase, thereby blocking transcription initiation?
(A) Tetracycline
(B) Chloramphenicol
(C) Rifampicin
(D) Streptomycin

2006

**145.** In eukaryotic cells, which specialized RNA polymerase is responsible for synthesizing all messenger RNA (mRNA) precursors?
(A) RNA Polymerase I
(B) RNA Polymerase II
(C) RNA Polymerase III
(D) Mitochondrial RNA Polymerase

2014

**146.** Eukaryotic RNA Polymerase I is strictly localized to the nucleolus. What is its primary transcriptional target?
(A) Pre-mRNAs
(B) tRNAs and 5S rRNA
(C) The large 45S ribosomal RNA precursor
(D) Small nuclear RNAs (snRNAs)

2003

**147.** Eukaryotic core promoters targeted by RNA Polymerase II frequently contain a consensus sequence located approximately at position -25 to -30. What is this element?
(A) The Pribnow box
(B) The TATA box
(C) The Initiator (Inr) element
(D) The Downstream Promoter Element (DPE)

2018

**148.** Which general transcription factor (GTF) specifically recognizes and binds to the TATA box to initiate the assembly of the pre-initiation complex (PIC)?
(A) TFIIA
(B) TFIIB
(C) TFIID
(D) TFIIH

2021

**149.** The TATA-binding protein (TBP) is a crucial subunit of which multi-protein general transcription factor?
(A) TFIID
(B) TFIIA
(C) TFIIE
(D) TFIIF

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2016

**150.** Eukaryotic TFIIH is a complex, multifaceted transcription factor. What are its two critical enzymatic functions during transcription initiation?
(A) DNA polymerase and DNA ligase activities
(B) RNA synthesis and RNA cleavage activities
(C) DNA helicase activity and CTD kinase activity
(D) Histone acetylation and deacetylation activities

2021

**151.** Promoter clearance by RNA Polymerase II in eukaryotes requires the extensive phosphorylation of which specific region of the polymerase?
(A) The primary catalytic site located within the core enzyme complex
(B) The N-terminal tail of the largest subunit of the polymerase enzyme
(C) The specialized sigma factor analogue required for initial recognition
(D) The C-terminal domain (CTD) of Rpb1

2021

**152.** Enhancers are critical cis-acting regulatory elements in eukaryotic transcription. Which characteristic strictly defines their function?
(A) Position- and orientation-independent function
(B) Mandatory location immediately adjacent to the core promoter region
(C) Coding for repressor proteins that silence all gene transcription
(D) Serving directly as origins for the initiation of DNA replication

2001

**153.** How do enhancer elements physically interact with the core promoter machinery located thousands of base pairs away?
(A) Altering the local salt concentration along the physical DNA backbone
(B) Transcribing specific regulatory microRNAs to target the promoter
(C) Unwinding the entire sequence of intervening DNA between the elements
(D) DNA looping to bring activator proteins into spatial proximity

2007

**154.** Eukaryotic RNA Polymerase III primarily targets genes encoding which essential cellular components?
(A) Synthesis of all functional and informational messenger RNA molecules
(B) Processing of the long pre-ribosomal 45S RNA precursor transcript
(C) tRNAs, 5S rRNA, and small non-coding RNAs
(D) Transcription of regulatory long non-coding RNAs within the nucleus

NODIA

2021

**155.** The alpha-alpha-amanitin toxin, produced by the death cap mushroom, is a potent inhibitor of transcription. Which polymerase is highly sensitive to very low concentrations of this toxin?
(A) RNA Polymerase I
(B) RNA Polymerase II
(C) RNA Polymerase III
(D) Bacterial RNA Polymerase

2015

**156.** Consider the following statements regarding eukaryotic transcription:
I. Enhancers can function effectively whether located upstream or downstream of a gene.
II. Basal transcription factors are absolutely required for basal levels of transcription.
III. RNA Polymerase I synthesizes the 5S rRNA transcript.
IV. Mediator complex acts as a coactivator bridge between enhancer-bound factors and the PIC.
Which of the above statement(s) is/are INCORRECT?
(A) I and II
(B) III only
(C) III and IV
(D) II and IV

2025

**157.** Maturation of eukaryotic pre-mRNA begins with the co-transcriptional addition of a specific structure at the 5' end. What is this modification?
(A) A 7-methylguanosine cap
(B) A polyadenylate tail
(C) A specific CCA trinucleotide
(D) A spliceosome assembly factor

2002

**158.** What type of unique linkage connects the 5' cap to the initial nucleotide of the nascent pre-mRNA transcript?
(A) A standard 5'-to-3' phosphodiester bond
(B) A 3'-to-5' phosphodiester bond
(C) A 5'-to-5' triphosphate bridge
(D) An N-glycosidic bond

2025

**159.** Which primary functional advantage is conferred to the mRNA molecule by the presence of the 5' cap?
(A) Protects mRNA from 5' exonucleases and assists in ribosome binding
(B) Dictates the precise sequence of amino acids to be translated into protein
(C) Provides a mandatory docking site for the assembly of the spliceosome
(D) Promotes the direct and rapid transport of mRNA into the nucleolar region

2011

**160.** The 3' end of most eukaryotic mature mRNAs is characterized by the presence of a poly(A) tail. How is this tail synthesized?
(A) Direct transcription from a genomic poly(T) sequence in the DNA template
(B) Post-transcriptional addition by poly(A) polymerase
(C) Splicing onto the transcript from a distinct and pre-existing precursor RNA
(D) Enzymatic addition during the translation process by the ribosome complex

2010

**161.** Cleavage and polyadenylation of the pre-mRNA transcript at the 3' end typically occurs 10-30 nucleotides downstream of which highly conserved signal sequence?
(A) AAUAAA
(B) UAGUAA
(C) GU-rich element
(D) TATAAT

2007

**162.** The process of pre-mRNA splicing fundamentally involves the removal of which non-coding sequences?
(A) Exons
(B) Promoters
(C) Enhancers
(D) Introns

2001

**163.** The spliceosome is a massive macromolecular complex responsible for catalyzing splicing. What are its core catalytic components?
(A) Specialized DNA helicases and ligases that rearrange the RNA sequence
(B) Complexes of proteases and ribozymes that degrade non-coding segments
(C) Small nucleolar RNAs (snoRNAs) that guide chemical modifications of RNA
(D) snRNPs such as $U_1$, $U_2$, $U_4$, $U_5$, and $U_6$

2022

**164.** The chemistry of nuclear pre-mRNA splicing occurs via two sequential transesterification reactions. The first reaction involves a nucleophilic attack by the 2'-OH group of which specific conserved residue?
(A) Adenine in the intronic branch point sequence
(B) A guanine residue located precisely at the 5' splice site junction
(C) A cytosine residue located precisely at the 3' splice site junction
(D) A uracil residue located within the intronic polypyrimidine tract sequence

2016

**165.** What transient, intermediate structure is formed by the intron immediately following the first transesterification step of splicing?
(A) A lariat structure
(B) A perfect linear double helix
(C) A closed circular RNA molecule
(D) A cruciform RNA

2012

**166.** Alternative splicing represents a crucial regulatory mechanism because it allows a cell to:
(A) Dramatically increase the overall rate of transcription for essential genes
(B) Circumvent the standard requirement for a 5' cap and a poly(A) tail
(C) Repair severe DNA damage post-transcriptionally within the coding region
(D) Produce multiple protein isoforms from a single locus

2004

**167.** Group I and Group II introns differ fundamentally from spliceosome-dependent introns in what striking functional aspect?
(A) Strictly requiring the hydrolysis of ATP for their removal from the transcript
(B) Composed exclusively of a large number of highly modified protein subunits
(C) Entirely selF-splicing ribozymes
(D) Splicing adjacent exons in a backward orientation relative to the transcript

2017

**168.** Which snRNP is specifically responsible for recognizing and directly base-pairing with the conserved 5' splice site of the pre-mRNA?
(A) $U_1$
(B) $U_2$
(C) $U_4$
(D) $U_6$

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2008
**169.** Out of the 64 possible codons, how many specify amino acids (sense codons)?
(A) 60
(B) 61
(C) 63
(D) 64

2014
**170.** Which feature of the genetic code indicates that a single amino acid may be encoded by more than one specific codon?
(A) Universality
(B) Non-overlapping nature
(C) Unambiguous nature
(D) Degeneracy (Redundancy)

2005
**171.** The canonical start codon, which establishes the precise reading frame and encodes methionine, is:
(A) AUG
(B) UAA
(C) UGA
(D) GUG

2023
**172.** Which of the following correctly lists the three standard stop (termination) codons?
(A) UAA, UAG, UGA
(B) UAA, UAC, UAG
(C) AUG, UAG, UGA
(D) UAA, UGA, UGG

2024
**173.** The "wobble hypothesis," proposed by Francis Crick, mathematically and structurally explains which phenomenon?
(A) Base-pairing relaxation at the third codon position
(B) Inherent instability of the interaction between the ribosome and mRNA
(C) Continuous reading of the genetic code without the use of punctuation
(D) Tendency of RNA and DNA polymerases to slip during the process of transcription

2008
**174.** If the first position of a tRNA anticodon (5' end) is the modified base inosine (I), it can successfully wobble-pair with which bases at the 3' end of the mRNA codon?
(A) Uracil, Cytosine, or Adenine
(B) Only Adenine
(C) Guanine or Cytosine
(D) Only Uracil

2005
**175.** Codon usage bias describes a biological phenomenon where:
(A) Mutations that occur exclusively within the third position of each codon
(B) Unequal frequency of synonymous codon usage
(C) Ribosomes that preferentially translate genes containing a high stop codon density
(D) tRNAs that fail to discriminate between chemically similar amino acid side chains

2004
**176.** Which fundamental rule ensures that the translation of an mRNA sequence produces a singular, specific polypeptide without frame-shifting confusion?
(A) The code exhibits a high degree of degeneracy for most amino acids
(B) The code is essentially universal across almost all known organisms
(C) Strict non-overlapping and continuous reading
(D) The code contains precisely three different signals for peptide termination

2024
**177.** Although highly conserved, the genetic code contains a few rare exceptions to universality. In human mitochondria, the codon UGA, normally a stop codon, is reassigned to encode which amino acid?
(A) Tryptophan
(B) Selenocysteine
(C) Arginine
(D) Methionine

NODIA

2010
**178.** What is the theoretical minimum number of distinct tRNAs required to translate all 61 sense codons in an organism?
(A) 20
(B) 31
(C) 61
(D) 64

2017
**179.** A single point mutation changes the mRNA codon UAU (Tyr) to UAA (Stop). What specific type of mutation has occurred?
(A) Nonsense mutation
(B) Missense mutation
(C) Silent mutation
(D) Frameshift mutation

2019

**180.** Consider the following statements concerning the genetic code:

I. The code is degenerate but strictly unambiguous.

II. The reading frame is established uniquely by the poly-A tail.

III. Wobble base pairing involves the 3' base of the anticodon and the 5' base of the codon.

IV. Selenocysteine is encoded by specific UGA codons dependent on contextual SECIS elements.

V. A continuous code implies the absence of untranslated "comma" nucleotides between codons.

Which of the above statement(s) is/are correct?

(A) I, IV, V
(B) I, II, III
(C) II, III, IV
(D) III, IV, V

2010

**181.** The prokaryotic ribosome, characterized by a sedimentation coefficient of 70S, is composed of which two primary subunits?

(A) 40S and 30S
(B) 50S and 30S
(C) 60S and 40S
(D) 50S and 20S

2005

**182.** Which specific ribosomal RNA molecule is an integral component of the prokaryotic small (30S) subunit?

(A) 5S rRNA
(B) 23S rRNA
(C) 28S rRNA
(D) 16S rRNA

2024

**183.** In eukaryotes, the intact, translationally active ribosome has a sedimentation coefficient of:

(A) 70S
(B) 80S
(C) 100S
(D) 120S

2001

**184.** The catalytic site responsible for peptide bond formation (peptidyl transferase center) is located within which ribosomal component?

(A) The small subunit rRNA (e.g., 16S)
(B) The highly conserved ribosomal protein L2
(C) The initiation factor complex
(D) The large subunit rRNA (e.g., 23S)

2024

**185.** Aminoacyl-tRNA synthetases are essential enzymes that perform what critical translational function?

(A) Splicing all pre-tRNA transcripts within the nucleus before their export
(B) Attaching the correct amino acid to its cognate tRNA
(C) Modifying individual nitrogenous bases within the tRNA anticodon loop region
(D) Catalyzing the final peptide bond formation at the active site of the ribosome

2003

**186.** The charging of a tRNA molecule by its specific aminoacyl-tRNA synthetase is an endergonic process driven by the hydrolysis of:

(A) GTP
(B) ATP
(C) CTP
(D) UTP

2011

**187.** Which specific intermediate is formed during the two-step activation and charging reaction catalyzed by aminoacyl-tRNA synthetase?

(A) Aminoacyl-GMP intermediate formed during the initial activation step
(B) A temporary peptide intermediate linked to the enzyme's catalytic site
(C) An RNA-protein hybrid complex that stabilizes the reactive amino acid
(D) Aminoacyl-AMP (aminoacyl-adenylate)

2022

**188.** Many aminoacyl-tRNA synthetases possess a proofreading (editing) mechanism to ensure extremely high fidelity. What does this editing function typically do?

(A) Recognizing and rapidly degrading all misfolded tRNA molecules in the cell
(B) Hydrolyzing the ester bond of mischarged tRNAs
(C) Cleaving the mRNA transcript if a premature stop codon is detected by the ribosome
(D) Actively repressing the transcription of all faulty aminoacyl-tRNA synthetase genes

2011

**189.** The functional ribosome contains three distinct tRNA binding sites. What is the correct spatial sequence of these sites in the direction of mRNA translation?

(A) A site → P site → E site
(B) E site → P site → A site
(C) P site → A site → E site

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(D) A site → E site → P site

2015

**190.** During active elongation, the growing polypeptide chain is physically anchored to a tRNA located primarily in which ribosomal site?
(A) E site
(B) P site
(C) A site
(D) Factor binding center

2018

**191.** The large eukaryotic ribosomal subunit (60S) contains multiple rRNA molecules. Which list correctly identifies them?
(A) 28S, 5.8S, and 5S
(B) 23S and 5S
(C) 18S, 5.8S, and 5S
(D) 28S and 18S

2018

**192.** Which statement accurately describes the relationship between a ribosome's protein and RNA components?
(A) Proteins constitute the catalytic core, while RNA provides a protective structural shell
(B) Ribosomes are composed entirely of proteins; rRNA is a distinct non-ribosomal transcript
(C) rRNA forms the catalytic core, while proteins provide stability
(D) Proteins and RNA contribute equally to the peptidyl transferase activity of the complex

2025

**193.** In prokaryotic translation initiation, the small 30S subunit precisely localizes the start codon by utilizing the Shine-Dalgarno sequence. Where is this sequence located?
(A) On mRNA, 5 to 10 nucleotides upstream of AUG
(B) Within the sequence of the 16S rRNA component of the small subunit
(C) On the initiator tRNA molecule itself at the base of the anticodon loop
(D) Within the primary promoter element that dictates transcription initiation

2025

**194.** The Shine-Dalgarno sequence on the mRNA base-pairs with a complementary pyrimidine-rich sequence located on which ribosomal component?
(A) The 3' end of the 16S rRNA
(B) The 5' end of the 23S rRNA
(C) The anti-codon of the initiator tRNA
(D) Initiation factor IF3

2002

**195.** In E. coli, the initiator tRNA strictly carries which specialized amino acid derivative?
(A) Methionine
(B) N-formylmethionine (fMet)
(C) Pyrrolysine
(D) Selenocysteine

2009

**196.** Which prokaryotic initiation factor is responsible for physically preventing the premature association of the 50S subunit with the 30S subunit?
(A) $IF_1$
(B) $IF_2$
(C) IF3
(D) EF-Tu

2023

**197.** Initiation of eukaryotic translation typically follows a scanning model. The pre-initiation complex binds to the 5' cap and scans downstream to locate the start codon embedded within which consensus sequence?
(A) Shine-Dalgarno sequence
(B) TATA box
(C) Pribnow box
(D) Kozak sequence

2018

**198.** During the elongation cycle, which factor delivers the next appropriate aminoacyl-tRNA to the A-site of the ribosome in a GTP-dependent manner?
(A) EF-Tu (prokaryotes) / $eEF_1A$ (eukaryotes)
(B) EF-G (prokaryotes) / $eEF_2$ (eukaryotes)
(C) $IF_2$
(D) Release Factor 1 ($RF_1$)

2023

**199.** What fundamental catalytic event occurs immediately after the correct aminoacyl-tRNA is accommodated in the A-site?
(A) Translocation of the entire ribosome complex along the mRNA transcript
(B) Enzymatic hydrolysis of the final mRNA transcript to terminate synthesis
(C) Spontaneous dissociation of the empty tRNA molecule from the ribosome
(D) Peptide bond formation via nucleophilic attack

NODIA

2004

**200.** Following peptide bond formation, the ribosome must shift exactly one codon further along the mRNA. This process, termed translocation,

requires energy and which specific elongation factor?
(A) EF-Tu
(B) EF-Ts
(C) EF-G
(D) RF3

2017

**201.** Termination of translation occurs when a stop codon enters the A-site. What physically recognizes this stop codon?
(A) Protein release factors (RFs) mimicking tRNA
(B) A specialized 'stop' tRNA molecule which lacks an attached amino acid
(C) The 16S rRNA sequence itself purely through steric hindrance of the site
(D) The enzymatic peptidyl transferase center located within the large subunit

2018

**202.** In E. coli, $RF_1$ specifically recognizes which termination codons?
(A) UAA and UAG
(B) UAA and UGA
(C) UAG and UGA
(D) All three stop codons

2023

**203.** The peptidyl transferase reaction catalyzed by the ribosome is chemically classified as what type of reaction?
(A) Transesterification forming an amide bond
(B) Phosphorylation of the terminal amino acid side chain residues
(C) Oxidation-reduction reaction occurring between adjacent subunits
(D) Methylation of the nitrogenous base at the C-terminus of the chain

2014

**204.** Which common antibiotic specifically inhibits prokaryotic translation by binding reversibly to the 30S subunit and blocking the binding of aminoacyl-tRNA to the A-site?
(A) Penicillin
(B) Tetracycline
(C) Chloramphenicol
(D) Erythromycin

2023

**205.** The E. coli lactose (lac) operon provides a classic model for negative inducible regulation. In the absence of lactose, what is the default state of the operon?
(A) Actively transcribed at basal levels throughout the cell cycle
(B) Repressed by the binding of CAP to the specific promoter region
(C) Activated by the stalling of RNA polymerase at the start codon
(D) Repressed by LacI binding to the operator

2025

**206.** The primary natural inducer of the lac operon, produced by the low-level action of beta-galactosidase, is:
(A) Lactose
(B) Glucose
(C) Allolactose
(D) Galactose

2015

**207.** Which gene within the lac operon explicitly encodes the enzyme beta-galactosidase?
(A) lacZ
(B) lacY
(C) lacA
(D) lacI

2005

**208.** The lac operon is also subject to positive control via catabolite repression. Which small effector molecule must accumulate to facilitate this positive control?
(A) ATP
(B) Cyclic AMP (cAMP)
(C) GMP
(D) Lactose

2022

**209.** Under which specific environmental conditions will the transcription of the lac operon be maximal?
(A) High glucose, high lactose
(B) High glucose, low lactose
(C) Low glucose, high lactose
(D) Low glucose, low lactose

2008

**210.** When cAMP levels are high, the cAMP-CAP complex binds to a specific site near the lac promoter. What is the direct functional consequence of this binding?
(A) It enzymatically degrades the LacI repressor protein in the cytoplasm
(B) It forcibly unwinds the DNA double helix at the structural genes
(C) It acts as a helicase to initiate DNA replication of the operon region
(D) Recruits and stabilizes RNA polymerase at the promoter

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2023

**211.** The E. coli tryptophan (trp) operon exemplifies negative repressible regulation. How does the presence of abundant exogenous tryptophan affect the system?
(A) Tryptophan acts as a co-repressor for the Trp repressor
(B) Tryptophan acts as an inducer, removing the repressor from the operator
(C) Tryptophan binds directly to RNA polymerase, halting all gene transcription
(D) Tryptophan downregulates the synthesis of cAMP effector molecules in the cell

2009

**212.** In addition to simple repression, the trp operon utilizes a secondary, sensitive regulatory mechanism that responds directly to the concentration of charged tRNA-Trp during transcription. What is this process termed?
(A) Catabolite repression
(B) Attenuation
(C) Anti-termination
(D) Allosteric inhibition

2016

**213.** The mechanism of attenuation relies crucially on the tight coupling of which two processes in prokaryotes?
(A) Transcription and replication
(B) Translation and splicing
(C) Replication and repair
(D) Transcription and translation

2002

**214.** In the following question, a statement of Assertion (A) is followed by a statement of Reason (R). Choose the correct option.
**Assertion (A):** During trp operon attenuation in low tryptophan conditions, the ribosome stalls at adjacent Trp codons in the leader transcript.
**Reason (R):** This stalling forces the nascent RNA to fold into an alternative anti-terminator hairpin, allowing transcription to proceed into the structural genes.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2007

**215.** If a mutation completely inactivates the lacI gene (repressor knock-out), what transcriptional phenotype will the lac operon exhibit?
(A) Uninducible state where the operon is permanently shut off
(B) Constitutive expression in the absence of lactose
(C) Hypersensitivity to glucose repression exclusively
(D) Complete reversal of the standard structural gene sequence order

2019

**216.** Consider the following statements concerning prokaryotic operon regulation:
I. The lac repressor physically blocks the path of RNA polymerase.
II. Attenuation only occurs in repressible operons and never in inducible systems.
III. cAMP levels are inversely proportional to glucose levels.
IV. The trp leader sequence contains two consecutive tryptophan codons.
V. CAP relies exclusively on GTP for activation.
Which of the above statement(s) is/are correct?
(A) I, II, III
(B) I, III, IV
(C) II, IV, V
(D) III, IV, V

2023

**217.** A spontaneous point mutation substitutes a purine base for another purine base (e.g., Adenine to Guanine). What is the specific classification of this mutation?
(A) Transition
(B) Transversion
(C) Frameshift
(D) Inversion

2003

**218.** Which structural alteration in DNA represents a transversion mutation?
(A) Cytosine to Thymine
(B) Adenine to Guanine
(C) Uracil to Cytosine
(D) Guanine to Cytosine

NODIA

2012

**219.** The spontaneous deamination of 5-methylcytosine within a DNA sequence produces which specific nitrogenous base, leading to a hotspot for transition mutations?
(A) Thymine
(B) Uracil
(C) Adenine
(D) Guanine

2003

**220.** Intercalating agents, such as ethidium bromide and proflavine, typically induce which class of genetic mutation?
(A) Silent single base transitions within the coding region
(B) Large-scale chromosomal translocations between chromosomes
(C) Massive double-strand DNA breaks throughout the genome
(D) Insertions or deletions leading to frameshifts

2011

**221.** An uncorrected insertion of precisely one nucleotide into the coding sequence of a gene inevitably results in:
(A) A synonymous substitution that does not change the amino acid
(B) Frameshift mutation altering all downstream amino acids
(C) A missense mutation strictly confined to a single amino acid residue
(D) Enhanced affinity of the promoter for the RNA polymerase enzyme

2023

**222.** The highly mutagenic base analog 5-bromouracil (5-BU) typically mimics thymine. However, its tendency to undergo tautomeric shifts frequently causes it to incorrectly pair with which base during replication?
(A) Adenine
(B) Cytosine
(C) Guanine
(D) Uracil

2008

**223.** What is the fundamental molecular consequence of a nonsense mutation occurring early in a gene's coding sequence?
(A) The synthesis of a hyperactive protein variant with increased activity
(B) Production of a truncated, non-functional polypeptide
(C) Alteration of a single catalytic residue without changing protein length
(D) Complete failure of RNA polymerase to recognize the initial promoter site

2023

**224.** Which physical agent is highly ionizing, capable of deeply penetrating tissues, and routinely causes massive DNA damage including widespread double-strand breaks?
(A) Gamma rays and X-rays
(B) Ultraviolet (UV) light at 260 nm
(C) Microwave radiation
(D) Infrared radiation

2006

**225.** Tautomeric shifts involve the spontaneous, transient rearrangement of protons on a nitrogenous base. For instance, the transition of the amino form of adenine to its rare imino form allows it to mispair with:
(A) Cytosine
(B) Thymine
(C) Guanine
(D) Uracil

2006

**226.** How does nitrous acid act as a potent chemical mutagen?
(A) Deaminates cytosine to uracil and adenine to hypoxanthine
(B) Induces the formation of pyrimidine dimers via UV energy
(C) Forces the intercalation of molecules into the DNA double helix
(D) Causes complete depurination of the DNA sugar-phosphate backbone

2007

**227.** A mutation that replaces an amino acid with another amino acid of similar chemical properties (e.g., leucine to isoleucine), often resulting in little to no functional impairment, is broadly termed a:
(A) Conservative missense mutation
(B) Lethal mutation
(C) Non-conservative missense mutation
(D) Nonsense mutation

NODIA

2022

**228.** Which physical agent is highly ionizing, capable of deeply penetrating tissues, and routinely causes massive DNA damage including widespread double-strand breaks?
(A) Gamma rays and X-rays
(B) Ultraviolet (UV) light at 260 nm
(C) Microwave radiation
(D) Infrared radiation

2005

**229.** Extrachromosomal inheritance displays which distinct pattern of transmission in animals and most plants?
(A) Uniparental maternal inheritance
(B) Mendelian biparental inheritance
(C) Strict paternal inheritance
(D) Random independent assortment

2001

**230.** The typical human mitochondrial genome (mtDNA) is structurally organized as:
(A) A linear, single-stranded DNA molecule carrying genetic information
(B) A highly fragmented and multi-chromosomal mitochondrial genome
(C) Small, circular, double-stranded DNA (16.5 kb)
(D) A giant, circular RNA molecule that acts as the primary genome

2005

**231.** Bacterial plasmids are defined as:
(A) Integrated segments of the primary circular bacterial chromosome
(B) Autonomous, selF-replicating extrachromosomal DNA
(C) Misfolded proteins capable of transmitting hereditary traits
(D) Viral genomes that have become permanently stalled in the lytic cycle

2025

**232.** Plasmid incompatibility refers to which specific biological phenomenon?
(A) The inherent inability of a plasmid to replicate within a eukaryotic cell
(B) Inability of related plasmids to coexist in one cell line
(C) The destruction of the host cell wall mediated by plasmid-encoded toxins
(D) The failure of specific plasmids to undergo horizontal genetic transfer

2008

**233.** Which class of bacterial plasmids encodes the genetic information necessary to synthesize conjugation pili and transfer DNA to recipient cells?
(A) F plasmids (Fertility)
(B) R plasmids (Resistance)
(C) Col plasmids (Colicinogenic)
(D) Degradative plasmids

2024

**234.** Mitochondrial genomes possess exceptionally high mutation rates compared to nuclear DNA. What is the primary cause?
(A) Lack of histones and proximity to reactive oxygen species
(B) Frequent and highly error-prone homologous recombination events in DNA
(C) Integration of highly unstable mobile retrotransposable elements in DNA
(D) Spontaneous depyrimidination by mitochondrial proteases in the matrix

2012

**235.** An individual cell may contain a mixture of normal and mutated mitochondrial genomes, leading to variable disease expressivity. What is this state termed?
(A) Heteroplasmy
(B) Homoplasmy
(C) Polyploidy
(D) Aneuploidy

2016

**236.** The copy number of a bacterial plasmid is fundamentally controlled by:
(A) Regulatory elements at the origin of replication (oriV)
(B) The total physical size of the host cell's primary chromosome
(C) The presence of heavy metals in the surrounding growth environment
(D) The specific sequence and length of the bacterial F-factor element

2005

**237.** Which human genetic disease is a classic example of a mitochondrially inherited disorder?
(A) Cystic Fibrosis
(B) Huntington's Disease
(C) Phenylketonuria (PKU)
(D) Leber Hereditary Optic Neuropathy (LHON)

2016

**238.** Certain specialized plasmids harbor genes that grant bacteria the ability to metabolize complex environmental toxins, such as toluene or salicylic acid. These are classified as:
(A) Ti plasmids
(B) Virulence plasmids
(C) Degradative plasmids
(D) Cryptic plasmids

2013

**239.** The genetic structure of the mammalian mitochondrial genome is highly compact. How many specific proteins are encoded by the human mtDNA?
(A) 13 critical components of oxidative phosphorylation
(B) Approximately 1,500 enzymes related to general cellular metabolism
(C) Over 3,000 regulatory proteins that manage mitochondrial function
(D) None; all mitochondrial proteins are strictly nuclear-encoded

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2010

**240.** What evolutionary theory explains the origin of extrachromosomal organelles like mitochondria and chloroplasts?
(A) The Endosymbiotic Theory
(B) The Spontaneous Generation Theory
(C) The Panspermia Hypothesis
(D) The Autogenous Hypothesis

2023

**241.** The simplest class of mobile genetic elements in bacteria, which encode only the transposase enzyme, are:
(A) Insertion sequences (IS elements)
(B) Composite transposons
(C) Retroviruses
(D) Non-LTR retrotransposons

2005

**242.** Insertion of a transposon into a new genomic location invariably produces which structural hallmark in the immediate target DNA sequence?
(A) A massive and localized deletion of the host's chromosomal DNA
(B) Short direct repeats flanking the inserted element
(C) Long terminal inverted repeats encompassing the whole chromosome
(D) Complete inversion of the host promoter following integration

2018

**243.** Bacterial composite transposons, such as Tn10, are uniquely characterized by which structural arrangement?
(A) Central auxiliary genes flanked by two insertion sequences
(B) They completely lack terminal inverted repeat sequences at their ends
(C) They utilize a mandatory RNA intermediate in order to mobilize
(D) They integrate strictly via homologous recombination with the host

2008

**244.** Barbara McClintock originally discovered transposable elements through her study of the Ac/Ds system in maize. In this system, how does the Ds (Dissociation) element differ from the Ac (Activator) element?
(A) Ds elements are non-autonomous and rely on Ac for mobilization
(B) Ds elements utilize an RNA intermediate while Ac elements utilize DNA
(C) Ds elements are autonomous and encode their own functional transposase
(D) Ds elements can only transpose into extrachromosomal plasmid DNA

NODIA

2011

**245.** Eukaryotic retrotransposons mobilize within the genome via which signature mechanism?
(A) Direct enzymatic excision and reinsertion ("cut-and-paste") mechanism
(B) Site-specific homologous crossover occurring between sister chromatids
(C) Independent viral budding and subsequent reinfection of the host cell
(D) RNA intermediate followed by reverse transcription

2022

**246.** The highly abundant Alu sequences found scattered throughout the human genome are classified as which specific type of mobile element?
(A) Long interspersed nuclear elements (LINEs)
(B) Short interspersed nuclear elements (SINEs)
(C) LTR-retrotransposons
(D) DNA transposons

2005

**247.** Unlike SINEs, LINEs (such as LINE-1 in humans) are generally considered autonomous elements because they:
(A) Do not require an RNA intermediate for their genomic mobilization
(B) Encode a functional reverse transcriptase and endonuclease
(C) Are continuously transcribed by the RNA Polymerase I enzyme complex
(D) Can physically bud from the host cell membrane as viral particles

2019

**248.** The replicative mechanism of transposition, as utilized by Tn3, leaves one copy of the transposon at the original site and inserts a new copy at the target site. This process requires an intermediate fused structure called a:
(A) Cointegrate
(B) Holliday junction
(C) D-loop
(D) Solenoid

2001

**249.** Which specific enzyme is essential for resolving the cointegrate intermediate back into two separate circular DNA molecules during replicative transposition?
(A) Transposase
(B) Resolvase

(C) DNA Ligase
(D) Topoisomerase

2011

**250.** Long Terminal Repeat (LTR) retrotransposons are structurally and evolutionarily most similar to which class of infectious agents?
(A) Bacteriophages
(B) Retroviruses
(C) Adenoviruses
(D) Viroids

2009

**251.** A transposon integrating directly into the coding sequence of an essential gene will most likely result in:
(A) Null mutation causing loss of gene function
(B) A dramatic increase in the target gene's overall transcriptional activity
(C) A completely silent mutation that does not affect the protein sequence
(D) Conversion of the target gene into a newly active transposable element

2004

**252.** The genetic phenomenon of "variegation," such as the spotted coloration observed in McClintock's maize kernels, is directly caused by:
(A) Accumulation of numerous point mutations over the lifespan of the organism
(B) Permanent and complete suppression of chromosomal crossing over events
(C) Viral infection leading to localized and programmed cell death in tissues
(D) Transposon insertion into a pigment gene and subsequent excision

2024

**253.** Tandemly repeated DNA sequences constitute a significant proportion of eukaryotic genomes. Minisatellites are also commonly referred to as:
(A) Variable Number Tandem Repeats (VNTRs)
(B) Short Tandem Repeats (STRs)
(C) Long Interspersed Elements (LINEs)
(D) Single Nucleotide Polymorphisms (SNPs)

2024

**254.** Microsatellites (SSRs) fundamentally differ from minisatellites primarily in which characteristic?
(A) SSRs have 1-6 bp repeats, while VNTRs have 10-100 bp
(B) SSRs are located exclusively within the telomeric regions of chromosomes
(C) SSRs do not exhibit any polymorphism between individuals in a population
(D) VNTRs are strictly transcribed into small regulatory RNA molecules in vivo

2012

**255.** The extremely high rate of spontaneous mutation leading to length variation in microsatellite tracts is primarily attributed to which specific molecular mechanism?
(A) Unequal crossing over during meiosis
(B) Massive radiation-induced double-strand breaks
(C) Slipped-strand mispairing (replication slippage) during DNA replication
(D) Targeted retrotransposon insertion

2016

**256.** If replication slippage occurs such that a loop forms in the newly synthesized daughter strand over a microsatellite region, what is the net result after the next replication cycle?
(A) An expansion (increase) in the number of tandem repeats
(B) A contraction (decrease) in the number of tandem repeats
(C) A transition mutation within the repeat sequence
(D) The complete deletion of the entire tandem array

2017

**257.** Which highly debilitating human neurological disorder is directly caused by the abnormal expansion of a trinucleotide repeat (CAG)?
(A) Sickle Cell Anemia
(B) Huntington's Disease
(C) Cystic Fibrosis
(D) Tay-Sachs Disease

2025

**258.** Tandem repeat arrays located at the extreme ends of linear eukaryotic chromosomes serve a specialized protective function. What are these specific arrays called?
(A) Centromeres
(B) Telomeres
(C) Nucleolar Organizers
(D) Origin Recognition Complexes

2024

**259.** What structural characteristic allows VNTRs to be exceptionally useful in classical DNA fingerprinting techniques?
(A) Highly variable restriction fragment lengths between individuals
(B) They are identical in total length across all members of a given species
(C) They strongly hybridize with bacterial

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plasmids under standard conditions
(D) They are completely devoid of any cytosine methylation at the CpG sites

2006

**260.** Which form of tandemly repeated DNA forms the primary structural constituent of functional centromeres in higher primates?
(A) Minisatellite DNA
(B) Alpha-satellite (alphoid) DNA
(C) Microsatellite DNA
(D) Ribosomal DNA arrays

2014

**261.** Why do mutations that alter the length of a microsatellite tract located strictly within deep intronic, non-regulatory regions generally persist without negative selection?
(A) Exert no significant phenotypic effect
(B) Immediately induce programmed cell death, removing them from the pool
(C) Invariably correct themselves during the development of the next generation
(D) Introns are rapidly converted into transcriptionally active coding sequences

2011

**262.** During forensic analysis, forensic scientists heavily utilize the multiplex PCR amplification of which specific type of highly polymorphic locus?
(A) Short Tandem Repeats (SNPs)
(B) Telomeric repeats
(C) Highly conserved housekeeping genes
(D) Entire mitochondrial genomes

2018

**263.** Unequal crossing over is a homologous recombination error that occurs during meiosis. It requires what specific prerequisite condition?
(A) Misalignment of chromatids along tandemly repeated DNA
(B) Complete absence of the synaptonemal complex during the meiotic prophase
(C) Direct infection by a retrovirus that integrates into the germline cells
(D) Prior massive exposure to high levels of ultraviolet radiation in the cell

2011

**264.** Fragile X syndrome is a quintessential example of a disorder caused by the expansion of a CGG repeat. Where is this critical repeat located in the affected FMR1 gene?
(A) In the 5'-UTR, leading to hypermethylation and silencing
(B) Deep within an intronic region between two highly conserved exons
(C) Directly in the primary active site of the globally encoded enzyme
(D) Within the 3' polyadenylation signal required for mRNA stability

2017

**265.** The classical Robin Holliday model of homologous recombination involves the formation of a cross-shaped intermediate structure. What defines this structure?
(A) Mobile junction involving reciprocal strand exchange between homologs
(B) A point where a single DNA strand completely disconnects from the backbone
(C) A triple-helix formation created by invading viral RNA within the nucleus
(D) The temporary covalent binding of histones to the active replication fork

2025

**266.** Which step immediately follows the initial single-strand nicks and subsequent strand invasion in the Holliday model?
(A) Spliceosome assembly
(B) Resolution of the junction
(C) Complete template degradation
(D) Branch migration

2021

**267.** In E. coli, the initiation of homologous recombination at double-strand breaks is primarily mediated by which potent multifunctional enzyme complex?
(A) RecBCD enzyme complex
(B) RecA protein
(C) RuvABC complex
(D) DNA Polymerase I

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2021

**268.** The RecBCD complex encounters a specific 8-base sequence (the Chi site). What is the immediate result?
(A) It generates a blunt end on both strands at the specific Chi site sequence
(B) It completely degrades the entire chromosome into individual nucleotides
(C) Preferential 5' strand degradation, creating a 3' overhang
(D) It circularizes the DNA fragment to facilitate subsequent plasmid formation

2001

**269.** The single-stranded 3' overhang generated by RecBCD is rapidly coated by which critical recombinase protein?
(A) SSB
(B) RecA
(C) LexA
(D) RuvA

2023

**270.** Branch migration during prokaryotic homologous recombination is actively driven by an ATP-dependent motor complex. Identify this complex.
(A) RuvAB
(B) RecBCD
(C) MutSL
(D) UvrAB

2002

**271.** To finalize the recombination process, the Holliday junction must be physically cleaved (resolved). Which specialized endonuclease performs this resolution step in E. coli?
(A) RecA
(B) RuvC
(C) EcoRI
(D) Cas9

2018

**272.** Depending on the plane of cleavage applied by RuvC to the Holliday junction, the resulting recombinant chromosomes will exhibit which two potential outcomes?
(A) Crossover (splice) or non-crossover (patch) products
(B) Complete deletion or massive duplication
(C) Transitions or transversions
(D) Lytic or lysogenic states

2010

**273.** The Double-Strand Break Repair (DSBR) model updates the classical Holliday model. A hallmark of the DSBR model is the formation of:
(A) Two adjacent Holliday junctions
(B) A single, non-migrating junction
(C) A massive D-loop without strand exchange
(D) An RNA intermediate

2001

**274.** In the initial stage of meiotic homologous recombination, the generation of the mandatory double-strand breaks is deliberately catalyzed by which specialized eukaryotic endonuclease?
(A) Spo11
(B) Dmc1
(C) Rad51
(D) Topoisomerase II

2005

**275.** Gene conversion is most commonly a direct result of which specific DNA repair process operating within the heteroduplex region?
(A) Mismatch Repair (MMR)
(B) Nucleotide Excision Repair
(C) Base Excision Repair
(D) Direct Reversal

2019

**276.** The D-loop (displacement loop) is a critical structural intermediate formed primarily during which specific phase of homologous recombination?
(A) Resolution
(B) Branch migration
(C) Initial single-strand invasion into a homologous duplex
(D) Following RuvC cleavage

2015

**277.** Bacterial transformation is defined strictly as the process by which a recipient cell:
(A) Takes up free extracellular DNA from the environment
(B) Receives new genetic material specifically via a bacteriophage vector
(C) Exogenously receives a plasmid through direct physical cell-to-cell contact
(D) Fuses completely with another bacterial cell to form a larger diploid

2015

**278.** Only cells in a specialized physiological state are capable of natural transformation. What is this state called?
(A) Conjugative
(B) Competent
(C) Lysogenic
(D) Virulent

2003

**279.** During the uptake of linear double-stranded DNA in natural transformation, what generally happens to the incoming DNA fragment?
(A) It enters entirely double-stranded and integrates as a large genetic cassette
(B) One strand is degraded, and a single strand enters the cytoplasm
(C) It is immediately circularized to form a new and autonomous plasmid unit
(D) It is immediately digested into individual nucleotides for nutrient use in cells

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2008

**280.** Bacterial conjugation, unlike transformation, fundamentally requires which mechanical condition?
(A) The mandatory presence of a viral intermediate to transfer the genetic data
(B) Naked DNA molecules freely suspended in the surrounding growth medium
(C) Heavy irradiation of the population to induce a state of cellular competency
(D) Physical cell-to-cell contact via a conjugative pilus

2021

**281.** During an $F^+ \times F^-$ mating event, what is the usual genetic outcome for the recipient ($F^-$) cell?
(A) Receives a single-stranded copy of F plasmid and becomes $F^+$
(B) It becomes a permanently and highly mutable cell within the population
(C) It receives only chromosomal genes and remains in the $F^-$ state throughout
(D) It suffers massive and irreversible DNA damage and subsequently dies

2005

**282.** During F-factor transfer, the DNA is nicked and physically unwound at a specific sequence known as:
(A) oriT (origin of transfer)
(B) oriC
(C) The promoters
(D) Ter sites

2012

**283.** What unique genetic state defines an Hfr (High frequency of recombination) strain of E. coli?
(A) It completely lacks the F-factor genetic sequence within its entire genome
(B) The F-factor exists as a small, autonomous, and hyper-replicating plasmid
(C) F-factor integrated into the host chromosome
(D) It possesses multiple distinct and independent F-plasmids within the cell

2006

**284.** In an interrupted mating experiment utilizing an Hfr donor and an $F^-$ recipient, the sequential, timed appearance of specific donor alleles in the recipient is primarily used to map:
(A) Relative linear order and distance of genes on the chromosome
(B) The exact physical location of the cell membrane in the donor bacterium
(C) The cumulative frequency of frameshift mutations across the whole population
(D) The precise physical size and diameter of the conjugative F-pilus structure

2017

**285.** In a typical Hfr x $F^-$ conjugation event, why does the recipient cell usually remain functionally $F^-$ following the transfer?
(A) The F-factor genes are immediately degraded by the recipient's restriction enzymes
(B) The transferred DNA sequence fails to undergo homologous recombination events
(C) The recipient lacks the ribosomes necessary to translate F-factor proteins in time
(D) Conjugation bridge breaks before the entire chromosome is transferred

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2009

**286.** Which DNA replication mechanism is exclusively utilized by the F-plasmid for transfer?
(A) Bidirectional Theta replication
(B) Rolling-circle replication
(C) Displacement-loop replication
(D) Semi-discontinuous Trombone replication

2017

**287.** Occasionally, the integrated F-factor in an Hfr cell undergoes imprecise excision, pulling adjacent chromosomal genes along with it. What is this modified plasmid called?
(A) An $F^+$ plasmid
(B) An F' (F-prime) plasmid
(C) An R plasmid
(D) A Ti plasmid

2007

**288.** The mating of an F' donor with an $F^-$ recipient cell leads to a state where the recipient becomes diploid for only specific genes. This state is termed a:
(A) Merodiploid (partial diploid)
(B) Complete autotetraploid
(C) Haploid mutant
(D) Lysogen

2015

**289.** Generalized transduction is mechanistically characterized by the accidental packaging of:
(A) Random host chromosome fragments packaged into a viral capsid
(B) Specifically the viral genome integrated alongside host resistance genes
(C) Exclusively ribosomal RNA molecules packaged within the capsid structure
(D) Specialized viral proteins integrated directly into the host genome sequence

2018

**290.** In generalized transduction, any gene from the donor bacterial chromosome has roughly an equal probability of being transferred. Why?
(A) Because the phage purposefully replicates the entire bacterial genome each cycle
(B) Host chromosome is shattered into phage-sized fragments and packaged
(C) Because the phage genome physically scrambles all available host DNA sequences
(D) Because only small bacterial plasmids are capable of being transferred in this way

2018

**291.** Specialized transduction can strictly transfer:
(A) Host genes located immediately adjacent to the prophage integration site
(B) Any random genetic locus located anywhere on the bacterial chromosome
(C) Only the antibiotic resistance markers present in the bacterial population
(D) Entire $F^-$ plasmids that are currently existing within the donor cell cytoplasm

2005

**292.** In the E. coli genome, the normal integration site for the lambda prophage (attB) is located specifically between which two operons?
(A) lac and trp
(B) his and leu
(C) ara and xyl
(D) gal and bio

2012

**293.** The integration of the lambda phage genome into the host chromosome requires which viral enzyme?
(A) Lambda Integrase (Int)
(B) Transposase
(C) Reverse Transcriptase
(D) RNA Polymerase

2001

**294.** A bacterial cell that stably harbors a dormant viral genome integrated into its chromosome is referred to as a:
(A) Competent cell
(B) Merodiploid
(C) Transformant
(D) Lysogen

2016

**295.** What specific trigger most commonly induces a lambda prophage to enter the active lytic cycle?
(A) SOS response to DNA damage, leading to viral repressor cleavage
(B) Exposure of the bacterial population to extreme and sudden cold shock
(C) Exposure to highly concentrated glucose levels within the growth medium
(D) Massive and rapid plasmid replication occurring within the host cell cytoplasm

2005

**296.** During the lambda phage lytic cycle, the decision to enter either lysis or lysogeny relies on which two regulatory proteins?
(A) cI (lambda repressor) and Cro
(B) LexA and RecA
(C) LacI and CAP
(D) Int and Xis

2010

**297.** In the context of specialized transduction, an aberrantly excised lambda phage genome may be:
(A) Defective, requiring a helper phage for replication and lysis
(B) Hyper-virulent towards all known strains of the host bacterial species
(C) A plaque-forming mutant that replicates faster than the standard wild-type
(D) Capable of immediately transforming into a free-living and autonomous bacterium

2002

**298.** The specific site-specific recombination event that excises the lambda prophage requires Int and:
(A) Cro
(B) cII
(C) Excisionase (Xis)
(D) RecA

2012

**299.** Recombination between viral genomes during mixed infections in $T_4$ phages yield unique structures described as:
(A) Heteroduplex joints
(B) Okazaki fragments
(C) Telomeric loops
(D) Solenoids

2009

**300.** If two genes exhibit a high frequency of cotransduction, what can be definitively concluded?
(A) They are located on entirely different chromosomes within the bacterial cell
(B) One specific gene actively regulates the transcription of the other gene
(C) Genes located close together, within a single

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phage DNA headful

(D) They possess identical nucleotide sequences across their entire coding regions

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# ANSWERS

**1.** (A) Transforming principle demonstration
Griffith identified the transforming principle through his in vivo experiments with Streptococcus pneumoniae. By injecting mice with a mixture of heat-killed virulent S strain and live non-virulent R strain, he observed that the mice died. The discovery that the R strain acquired the ability to produce a capsule from the dead S cells demonstrated bacterial transformation in a biological system.

**2.** (C) Proteases, RNases, and DNases
These researchers extended the work of Griffith by systematically destroying specific macromolecules in bacterial extracts using specialized enzymes. They utilized proteases to degrade proteins, RNases for RNA, and DNases for DNA. Transformation only ceased when DNA was degraded, providing definitive biochemical evidence that DNA, rather than protein or RNA, is the transforming principle responsible for carrying hereditary information.

**3.** (A) Phosphorus-32 and Sulfur-35
The Hershey-Chase experiment utilized radioactive isotopes to distinguish between viral components. Phosphorus-32 was chosen to label DNA because phosphorus is found in nucleic acids but not in most proteins. Conversely, Sulfur-35 was used to label proteins as sulfur is present in specific amino acids but entirely absent from the DNA molecular structure. This allowed tracking of the genetic material.

**4.** (D) RNA is the genetic material in TMV
Researchers demonstrated that RNA acts as the genetic material in Tobacco Mosaic Virus through reconstitution experiments. By separating and then combining hybrid viruses from the RNA and protein of different strains, they observed that the resulting progeny always matched the strain of the RNA component. This established that RNA, not protein, carries the genetic information for viral replication in plants.

**5.** (B) Competence
Bacterial transformation involves the direct uptake of exogenous DNA from the surrounding environment. For this to occur, bacteria must be in a specialized physiological state known as competence. This state involves the expression of specific proteins that facilitate DNA binding and transport across the cell membrane, allowing the successful integration of new genetic information into the bacterial chromosome or plasmid.

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**6.** (C) DNA was perceived as chemically too simple
During the early twentieth century, scientists believed proteins were the genetic carriers due to their structural complexity involving twenty different amino acids. DNA, composed of only four types of nucleotides, was dismissed as being too chemically simple to encode the vast diversity of life. Consequently, the findings of Avery and colleagues were initially met with significant skepticism from the community.

**7.** (D) Mechanical shearing by a laboratory blender
The Hershey-Chase experiment utilized a kitchen blender to provide mechanical shearing force. This physical process was essential to detach the empty viral protein shells, or ghosts, from the surfaces of the infected bacterial cells after the injection of genetic material. Subsequent centrifugation then separated the heavier bacteria from the lighter viral coats to determine which component entered the host.

**8.** (A) Type A RNA and Type A protein
Reconstitution experiments involving Tobacco Mosaic Virus demonstrated that the phenotypic traits and genetic composition of progeny viruses are determined solely by the parental RNA. When researchers combined RNA from type A with protein from type B, the resulting offspring contained only type A RNA and type A proteins. This confirmed that the protein coat does not carry hereditary instructions.

**9.** (B) The phosphate backbone is exclusive to nucleic acids among macromolecules
Phosphorus-32 is an ideal radioactive isotope for tracking nucleic acids because phosphorus is a fundamental constituent of the sugar-phosphate backbone in DNA and RNA. Proteins generally lack phosphorus, typically containing sulfur instead. This chemical distinction allowed Hershey and Chase to selectively label DNA,

ensuring that radioactivity detected inside infected bacteria originated specifically from the viral nucleic acid, not protein.

**10.** (B) RNase treatment completely abolished the transforming activity
Experimental findings indicated that the transforming principle was resistant to both proteolytic enzymes and ribonucleases. The addition of RNase did not destroy the ability of the smooth strain extract to transform rough cells. Only the application of deoxyribonuclease, which targets DNA, resulted in the loss of transforming activity. This demonstrated that RNA was not the genetic carrier for these bacteria.

**11.** (A) Transformation involves naked DNA, whereas transduction requires a viral vector
Transformation is characterized by the uptake of naked DNA directly from the extracellular environment into a competent bacterial cell. In contrast, transduction involves the transfer of bacterial DNA from one cell to another mediated by a viral vector, such as a bacteriophage. Both processes result in horizontal gene transfer but utilize different mechanisms for DNA delivery and protection from degradation.

**12.** (C) P-4, Q-3, R-2, S-1
Scientific progress in identifying genetic material involved key discoveries by several researchers. Griffith first described bacterial transformation, while Avery, MacLeod, and McCarty identified the transforming principle as DNA. Hershey and Chase proved viral DNA enters host cells using radioactive labeling. Finally, Fraenkel-Conrat demonstrated that RNA serves as the genetic material in certain viruses like the Tobacco Mosaic Virus.

**13.** (D) Adenine
Adenine is a specific purine base characterized by a double-ring heterocyclic structure. Chemically, it is identified as 6-aminopurine because it possesses an amino group attached to the sixth carbon atom of the purine ring. This base pairs with thymine in DNA and uracil in RNA through two hydrogen bonds, maintaining the stability of the double-stranded nucleic acid architecture.

**14.** (C) N-glycosidic bond
In the molecular structure of a nucleoside, a nitrogenous base is covalently attached to a pentose sugar. This specific linkage is known as an N-glycosidic bond. It forms between the C1' carbon of the ribose or deoxyribose sugar and either the $N_1$ nitrogen of a pyrimidine or the N9 nitrogen of a purine, creating a stable and functional unit.

**15.** (D) 5' carbon
A nucleotide consists of a nucleoside and at least one phosphate group. In standard biological nucleotides, the phosphate group is covalently bonded to the 5' carbon atom of the pentose sugar. This arrangement is crucial for the formation of the phosphodiester backbone, as the 5' phosphate of one nucleotide reacts with the 3' hydroxyl group of the next nucleotide.

**16.** (D) A methyl group at the C5 position
Thymine and uracil are both pyrimidine bases that are chemically similar. The primary distinction between them is the presence of a methyl group at the C5 position of the pyrimidine ring in thymine. Consequently, thymine is also referred to as 5-methyluracil. This modification increases the stability of DNA and helps distinguish it from RNA, which contains uracil.

**17.** (A) 3' and 5'
The structural integrity of a polynucleotide chain is maintained by phosphodiester linkages. These covalent bonds connect the 3' carbon atom of one pentose sugar to the 5' carbon atom of the adjacent sugar residue through a phosphate group. This repeating sugar-phosphate backbone provides a directional orientation to the DNA or RNA molecule, defined as the 5' to 3' polarity.

**18.** (A) Both A and R are true and R is the correct explanation of A.
RNA is notably more susceptible to alkaline hydrolysis than DNA due to its molecular structure. The ribose sugar in RNA contains a hydroxyl group at the 2' position, which is absent in the deoxyribose of DNA. Under basic conditions, this 2' hydroxyl group acts as a nucleophile, attacking the adjacent phosphodiester bond and causing the RNA backbone to fragment.

**19.** (A) Guanosine
A nucleoside is formed by the combination of a nitrogenous base and a pentose sugar. When the purine base guanine is linked to a ribose sugar, the resulting molecule is specifically named

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guanosine. If the sugar were deoxyribose, it would be called deoxyguanosine. Guanosine serves as a precursor for the synthesis of guanylate, which is a core component of RNA.

**20.** (D) A single-ring heterocyclic structure
Pyrimidines are a class of nitrogenous bases characterized by a single six-membered heterocyclic ring structure. This ring consists of four carbon atoms and two nitrogen atoms. The common pyrimidines found in nucleic acids are cytosine, thymine, and uracil. Unlike purines, which have a double-ring system, pyrimidines are smaller molecules that pair with purines to maintain a constant DNA diameter.

**21.** (B) Alternating pentose sugars and phosphate groups
The structural framework of nucleic acids like DNA and RNA consists of a repeating sugar-phosphate backbone. This backbone is formed by alternating molecules of pentose sugars and phosphate groups linked by phosphodiester bonds. The nitrogenous bases are attached to the sugars and project inward, but the backbone itself provides the physical stability and directional polarity required for genetic information storage.

**22.** (C) A nucleoside lacks a phosphate group
The primary chemical difference between a nucleoside and a nucleotide is the presence of a phosphate group. A nucleoside consists only of a nitrogenous base attached to a pentose sugar. When one or more phosphate groups are covalently bonded to the 5' carbon of the sugar, the molecule becomes a nucleotide, which serves as the basic building block of DNA.

**23.** (D) Pyrophosphate
During the process of polynucleotide synthesis, DNA or RNA polymerase adds a new nucleotide to the growing chain. The incoming nucleoside triphosphate is cleaved, releasing the two terminal phosphate groups as a single molecule called pyrophosphate. The energy released from the hydrolysis of this pyrophosphate molecule helps drive the polymerization reaction forward, ensuring the successful extension of the nucleic acid.

**24.** (B) I and III
Cytosine is a pyrimidine that contains a carbonyl group at the C2 position and an amino group at C4. During nucleic acid synthesis, purines are uniquely assembled directly onto the ribose sugar scaffold. In contrast, adenine forms only two hydrogen bonds with thymine, not three. Therefore, the statements concerning cytosine structure and purine synthesis are accurate while the bonding.

**25.** (B) B-DNA
B-DNA is the most common double-helical structure found in living cells under physiological conditions. It was the specific conformation described by Watson and Crick in their seminal model. This right-handed helix is characterized by a high degree of hydration and features well-defined major and minor grooves, which are essential for the binding of regulatory proteins to the DNA sequence.

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**26.** (B) It increases due to the hyperchromic effect
When a DNA molecule undergoes denaturation, the two complementary strands separate and the double-helical structure collapses. This transition leads to a significant increase in the absorption of ultraviolet light at 260 nm, a phenomenon known as the hyperchromic effect. This occurs because the stacked nitrogenous bases in the double helix absorb less light than the unstacked bases in single-stranded DNA.

**27.** (A) Guanine-Cytosine
The melting temperature of a DNA molecule reflects the amount of thermal energy required to separate its strands. This value is directly proportional to the percentage of guanine-cytosine base pairs. Guanine and cytosine are joined by three hydrogen bonds, whereas adenine and thymine have only two. The stronger triple bonding in GC pairs provides greater thermal stability to the helix.

**28.** (C) Left-handed helical rotation
Z-DNA is a unique structural variant of the DNA double helix characterized by a left-handed rotation. Unlike the standard right-handed B-DNA, Z-DNA possesses a zigzag-shaped sugar-phosphate backbone. It often forms in genomic regions with alternating purine and pyrimidine sequences, especially under high salt concentrations or when the DNA is under torsional stress, such as during active replication or transcription.

**29.** (D) Dehydrating conditions or low humidity
A-DNA is a structural conformation that typically forms when DNA is subjected to dehydrating conditions or low humidity. Like B-DNA, it is a right-handed helix, but it is shorter and wider, with base pairs that are tilted rather than perpendicular to the helical axis. This form is also commonly observed in RNA-DNA hybrids and double-stranded RNA regions within cells.

**30.** (C) B-DNA → A-DNA → Z-DNA
DNA can transition between different structural forms based on its environment. Under standard physiological hydration, it exists as B-DNA. If the hydration levels decrease, it may shift to the A-DNA conformation. In the presence of high salt concentrations and specific alternating GC sequences, the helix can undergo a radical change to the left-handed Z-DNA form. This sequence represents increasing structural.

**31.** (B) Hydrophobic interactions and van der Waals forces
The stability of the DNA double helix is significantly enhanced by base stacking interactions. These occur between the flat, hydrophobic surfaces of adjacent nitrogenous bases within the same strand. Driven by the hydrophobic effect and van der Waals forces, stacking reduces the exposure of the bases to the aqueous environment and contributes more to the overall helical stability than hydrogen bonding.

**32.** (B) 10.5
In the standard B-DNA model, the double helix is right-handed and relatively flexible. On average, there are approximately 10.5 base pairs for every complete 360-degree turn of the helix. This reflects a helical rise of about 3.4 Angstroms per base pair and a total pitch of 36 Angstroms. This configuration ensures optimal packing and accessibility of the genetic information.

**33.** (B) 20 Angstroms
The standard B-DNA double helix has a consistent diameter across its entire length. This width is approximately 20 Angstroms, or 2 nanometers. This uniformity is maintained because a double-ringed purine base always pairs with a single-ringed pyrimidine base. The consistent distance between the two sugar-phosphate backbones is a fundamental structural requirement for the stable and predictable architecture of the material.

**34.** (C) Base pairing and stacking re-establishment
Renaturation, or annealing, is the process by which separate complementary DNA strands reform a double helix. When a denatured DNA solution is cooled slowly, the strands collide and randomly form base pairs. If these initial matches are correct, the strands zip back together, stabilized by the re-establishment of hydrogen bonds and base stacking. This property is fundamental to molecular biology.

**35.** (A) Asymmetrical attachment of base pairs to the backbone
The major and minor grooves of the DNA double helix are a direct consequence of the molecular geometry. The nitrogenous bases are not attached to the sugar-phosphate backbone at a perfect 180-degree angle. This asymmetrical attachment results in two unequal spaces between the backbones as they wind around the central axis, creating deep major grooves and narrower minor grooves for interaction.

**36.** (D) P-2, Q-4, R-1, S-3
Different DNA conformations possess distinct structural and optical characteristics. A-DNA is a right-handed helix with 11 base pairs per turn, while B-DNA is the biological standard with 10.5 base pairs. Z-DNA is distinguished by its left-handed zigzag backbone. Denatured DNA, having separated strands, exhibits high UV absorbance. Correctly matching these traits is essential for understanding the physical properties of acids.

**37.** (B) rRNA
In a rapidly growing bacterial cell, ribosomal RNA is the most abundant type of RNA, comprising about 80 percent of the total cellular RNA content. This high concentration is necessary to support the vast number of ribosomes required for intensive protein synthesis. In contrast, messenger RNA and transfer RNA exist in much smaller proportions despite their critical roles in the translation.

**38.** (C) Transfer RNA
The secondary structure of transfer RNA is characterized by a distinctive cloverleaf pattern. This shape is formed by the folding of the single RNA strand into several double-helical stems and

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single-stranded loops, including the anticodon loop and the D-loop. This specific arrangement allows the tRNA to carry an amino acid and interact precisely with the ribosome and mRNA during translation.

**39.** (D) Small nuclear RNAs (snRNAs)
Small nuclear RNAs (snRNAs) are essential components of the spliceosome, the ribonucleoprotein complex responsible for removing introns from pre-mRNA and joining exons to produce mature mRNA. Major snRNAs, including $U_1$, $U_2$, $U_4$, $U_5$, and $U_6$, recognize splice sites and catalyze splicing. In contrast, snoRNAs modify rRNA, miRNAs regulate gene expression, and rRNAs form the structural and catalytic core of ribosomes.

**40.** (A) mRNA → tRNA → rRNA
In prokaryotic cells, different RNA types exhibit varying degrees of metabolic stability. Messenger RNA generally has the shortest average halF-life, often lasting only a few minutes, which allows the cell to rapidly adjust gene expression. Transfer RNA and ribosomal RNA are much more stable, lasting for hours or even multiple cell generations, as they are integral components of machinery.

**41.** (B) A CCA trinucleotide sequence
Mature transfer RNA molecules possess a highly conserved trinucleotide sequence at their 3' terminus, consisting of the bases cytosine, cytosine, and adenine. This CCA tail is the site where the specific amino acid is covalently attached by an aminoacyl-tRNA synthetase enzyme. While the rest of the tRNA molecule provides structural recognition, this terminal sequence is essential for the process.

**42.** (A) Both A and R are true and R is the correct explanation of A.
Eukaryotic cells exhibit a strict spatial separation between transcription, which occurs in the nucleus, and translation, which occurs in the cytoplasm. This compartmentalization necessitates extensive pre-mRNA processing, such as capping and splicing, before the transcript can be exported and translated. In prokaryotes, these processes are coupled in the same space, allowing translation to begin while the mRNA is synthesized.

**43.** (A) Base-pairing with target mRNAs to alter their translation or stability
Small regulatory RNAs in bacteria play a crucial role in post-transcriptional gene regulation. These molecules typically function by base-pairing with specific target messenger RNAs. This interaction can either inhibit translation by blocking the ribosomal binding site or promote mRNA degradation by recruiting RNases. This mechanism allows bacteria to rapidly modulate protein production in response to environmental changes without altering sequences.

**44.** (C) It pairs with the complementary codon sequence on the mRNA
The anticodon loop of a transfer RNA molecule is responsible for the precise decoding of the genetic message. It contains a triplet of nucleotides, known as the anticodon, that base-pairs with the complementary codon sequence on the messenger RNA. This interaction ensures that the specific amino acid carried by the tRNA is correctly incorporated into the growing polypeptide chain according to instructions.

**45.** (D) snoRNA
Small nucleolar RNAs are specialized regulatory molecules located within the nucleolus of eukaryotic cells. Their primary function is to guide the site-specific chemical modification of ribosomal RNA, such as 2'-O-methylation and pseudouridylation. By base-pairing with target rRNA sequences, snoRNAs recruit enzymes that modify the RNA, which is essential for the proper folding and function of the resulting ribosomal subunits.

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**46.** (C) A structural scaffold and a ribozyme
Ribosomal RNA is a multifunctional molecule that serves as both a structural scaffold and a catalytic agent. Within the ribosome, rRNA molecules organize the various protein subunits into a functional complex. Furthermore, the large subunit rRNA acts as a ribozyme, directly catalyzing the formation of peptide bonds between amino acids. This demonstrates that RNA can possess enzymatic activity essential for life.

**47.** (D) Forming perfect matches with target mRNAs in animals
MicroRNAs are key regulators of gene expression that typically induce silencing by binding to the 3' untranslated regions of target

mRNAs. In animals, microRNAs usually form imperfect matches with their targets, leading to the inhibition of translation rather than direct mRNA cleavage. This differs from plant microRNAs, which often exhibit near-perfect complementarity and trigger the immediate endonucleolytic degradation of.

**48.** (B) II only
RNA molecules are structurally diverse and can fold into complex three-dimensional shapes. Transfer RNA assumes a tertiary L-shaped conformation, and many RNA sequences form double-helical regions through intramolecular base pairing. Non-canonical interactions, such as G-U pairs, are common in these structures. The assertion that RNA sequences cannot form double-helical regions is incorrect, as these motifs are fundamental to.

**49.** (B) Peptide bonds connecting consecutive amino acid residues
The primary structure of a protein is defined as the unique linear sequence of amino acids in its polypeptide chain. This level of organization is stabilized exclusively by covalent peptide bonds, which connect the alpha-carboxyl group of one amino acid to the alpha-amino group of the next. The specific order of these residues ultimately determines how the protein will fold.

**50.** (C) I and III
The peptide bond is a specialized covalent linkage with several distinctive properties. Due to resonance, it possesses partial double-bond character, which prevents rotation around the C-N axis and keeps the bond planar. Most peptide bonds in proteins adopt a trans configuration to minimize steric interference between side chains. These characteristics are fundamental to the structured folding of the polypeptide backbone.

**51.** (A) Phi ($\phi$) and Psi ($\psi$) dihedral angles of the backbone
The Ramachandran plot is a fundamental tool in structural biology used to visualize the conformational freedom of the polypeptide backbone. It plots the dihedral angles, specifically Phi and Psi, around the alpha-carbon atoms. By identifying the sterically allowed regions for these angles, the plot predicts the possible secondary structures, such as alpha-helices and beta-sheets, that a protein can adopt.

**52.** (D) Alpha-helix
The alpha-helix is a common secondary structure motif in proteins, characterized by a tightly coiled, right-handed backbone. In this conformation, each turn of the helix contains approximately 3.6 amino acid residues. The structure is stabilized by hydrogen bonds formed between the carbonyl oxygen of one residue and the amide hydrogen of the residue four positions further along the chain.

**53.** (A) A chain reversal stabilized by an $i$ to $i+3$ hydrogen bond
A beta-turn is a common structural motif that allows a polypeptide chain to reverse its direction, typically at the protein surface. This sharp turn involves four amino acid residues and is stabilized by a hydrogen bond between the carbonyl oxygen of the first residue and the amide hydrogen of the fourth. These turns often contain glycine or proline to facilitate bending.

**54.** (A) Motif → Domain → Tertiary → Quaternary
Protein organization follows a hierarchical progression of increasing complexity. The primary sequence folds into motifs, which are simple combinations of secondary structures. These motifs assemble into domains, which are independent, stable units of the tertiary structure. Finally, multiple tertiary subunits may interact to form the quaternary structure. Understanding this sequence is essential for studying the relationship between structure and function.

**55.** (B) Hydrophobic effect burying nonpolar side chains in the interior
The tertiary structure of a globular protein is primarily stabilized by the hydrophobic effect. In an aqueous environment, nonpolar amino acid side chains tend to cluster together in the protein's interior to minimize their contact with water. This spontaneous process increases the entropy of the surrounding solvent and drives the folding of the polypeptide chain into its compact, functional shape.

**56.** (A) The spatial arrangement of multiple independent polypeptide subunits
Quaternary structure refers to the highest level of protein organization, involving the assembly of two or more independent polypeptide chains into a single functional complex. These individual chains, known as subunits, are held together

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primarily by non-covalent interactions, such as hydrogen bonds, ionic bridges, and hydrophobic forces. This level of structure allows for complex regulatory mechanisms like allosteric control.

**57.** (C) Proline
Proline is unique among the twenty standard amino acids because its side chain is covalently bonded to the nitrogen atom of the peptide backbone, forming a cyclic structure. This rigid arrangement severely restricts the rotational flexibility of the N-Ca bond, represented by the Phi angle. Consequently, proline occupies very limited regions on the Ramachandran plot compared to other amino acids.

**58.** (A) Glycine
Glycine is the simplest amino acid, containing only a single hydrogen atom as its side chain. This lack of a bulky R-group provides glycine with exceptional conformational flexibility, allowing it to adopt a wide range of Phi and Psi angles. As a result, glycine can occupy areas of the Ramachandran plot that are sterically forbidden for all other amino acids.

**59.** (C) Hydrogen bonding and hydrophobic interactions
Urea is a potent chemical denaturant that disrupts the stable three-dimensional structure of proteins. It functions primarily by interfering with the non-covalent interactions that stabilize tertiary and secondary structures, specifically hydrogen bonding and hydrophobic interactions. By competing for hydrogen bonds and increasing the solubility of nonpolar side chains, urea causes the protein to unfold into a random coil.

**60.** (A) P-2, Q-3, R-4, S-1
Each level of protein structure is maintained by specific molecular forces. The primary structure is stabilized by covalent peptide bonds, while the secondary structure relies on hydrogen bonds within the backbone. Tertiary structure is determined by various side-chain interactions, including hydrophobic forces and ionic bridges. Finally, quaternary structure involves non-covalent interactions between multiple subunits. Identifying these forces is key to biochemistry.

**61.** (B) The nucleoid is not enclosed by a distinct lipid bilayer membrane
In prokaryotic cells, the genetic material is organized into a region called the nucleoid. Unlike the eukaryotic nucleus, the nucleoid is not enclosed by a nuclear envelope or a lipid bilayer membrane. The circular DNA molecule resides directly in the cytoplasm, where it is associated with specific proteins that facilitate compaction, allowing transcription and translation to occur simultaneously.

**62.** (D) Negatively supercoiled circular DNA
Most bacterial chromosomes exist in a state of negative supercoiling, where the DNA double helix is slightly underwound compared to its relaxed state. This topological tension facilitates essential cellular processes, such as the separation of DNA strands during replication and transcription. Negative supercoils are essential for the compact organization of the large circular chromosome within the small cell volume.

**63.** (D) DNA Gyrase (Topoisomerase II)
DNA gyrase is a specialized type II topoisomerase in bacteria that actively introduces negative supercoils into the circular DNA molecule. This process requires the hydrolysis of ATP to provide the necessary energy. By breaking both strands of the double helix and passing another segment through the break, DNA gyrase maintains the underwound state of the chromosome for efficient replication.

**64.** (C) It decreases by increments of 2
The linking number represents the total number of times one DNA strand winds around another in a closed circular molecule. When DNA gyrase introduces negative supercoils, it catalyzes a reaction that reduces the linking number by increments of two. This change reflects the underwinding of the double helix, creating the torsional stress required to facilitate strand separation during processes.

**65.** (B) Supercoiled macrodomains or loops
The bacterial nucleoid is organized into a series of independent topological units known as supercoiled macrodomains or loops. These structures are formed by the interaction of DNA with nucleoid-associated proteins and anchoring to the cell membrane. This organization prevents the entire chromosome from becoming entangled and allows local changes in supercoiling to occur without affecting global DNA topology.

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**66.** (A) Both A and R are true and R is the correct explanation of A.
Topoisomerase I is an essential enzyme in bacteria that helps regulate DNA topology by relaxing negative supercoils. Unlike DNA gyrase, this enzyme does not require ATP. It functions by creating a transient single-strand break in the DNA, allowing the unbroken strand to pass through the gap before resealing the backbone. This mechanism prevents DNA from becoming excessively underwound.

**67.** (D) Nucleoid-associated proteins (NAPs) such as HU and IHF
Bacteria utilize a group of small, basic proteins called nucleoid-associated proteins, such as HU and IHF, to compact their DNA. These proteins function analogously to eukaryotic histones by binding to and bending the DNA, facilitating the formation of supercoiled loops. NAPs are essential for maintaining the structural integrity of the nucleoid and play a significant role in regulating gene expression.

**68.** (A) DNA Gyrase
Quinolone antibiotics, including ciprofloxacin, are powerful bactericidal agents that specifically target bacterial type II topoisomerases, primarily DNA gyrase and topoisomerase IV. By binding to the enzyme-DNA complex, these drugs prevent the religation of double-strand breaks created during the catalytic cycle. This results in the fragmentation of the bacterial chromosome, leading to the rapid inhibition of replication and cell.

**69.** (B) DNA Gyrase
As the replication fork advances, the unwinding of the DNA double helix introduces positive supercoiling ahead of the fork. If left unresolved, this torsional stress would eventually stall the replication machinery. DNA gyrase is the primary enzyme responsible for removing these positive supercoils by introducing negative supercoils, thereby maintaining the progress of the replication fork and ensuring efficient duplication.

**70.** (B) Decatenation of intertwined daughter chromosomes
Topoisomerase IV plays a critical role during the final stages of bacterial DNA replication. As the replication forks meet, the two circular daughter chromosomes become physically intertwined, a state known as catenation. Topoisomerase IV acts to decatenate these molecules by passing one entire double helix through a transient break in the other, allowing the daughter chromosomes to separate and segregate.

**71.** (A) DNA is underwound, resulting in negative supercoiling
The specific linking difference, or superhelical density, is a measure of the degree of supercoiling in a DNA molecule relative to its relaxed state. A value of approximately -0.06 indicates that the DNA is underwound by about 6 percent, resulting in negative supercoiling. This state is typical for bacterial plasmids and chromosomes, providing the necessary energy for strand separation.

**72.** (A) I and II
In DNA topology, twist refers to the winding of the two individual strands around each other to form the double helix. Writhe describes the coiling of the helical axis upon itself in space, such as the formation of supercoils. The linking number is the sum of twist and writhe. The assertions that define twist and writhe in the opposite.

**73.** (D) 147 bp of DNA wrapped around an octamer of core histones
The nucleosome is the fundamental structural unit of eukaryotic chromatin, enabling the massive compaction of DNA within the nucleus. It consists of a core particle containing approximately 147 base pairs of DNA wrapped nearly twice around a disk-shaped octamer of histone proteins. This "beads-on-a-string" arrangement is the first level of organization, which is further folded into higher-order.

**74.** (C) Two copies each of $H_2A$, $H_2B$, $H_3$, and $H_4$
The protein core of a nucleosome is a highly organized octamer composed of two copies each of four distinct histone proteins: $H_2A$, $H_2B$, $H_3$, and $H_4$. These histones are small, positively charged proteins that interact strongly with the negatively charged DNA backbone. The $H_3$ and $H_4$ subunits first form a tetramer, which then associates with two $H_2A$-$H_2B$ dimers.

**75.** (B) Compaction of nucleosomes into the 30 nm fiber
Histone $H_1$ is known as the linker histone because it binds to the DNA segment located between nucleosomes and to the core particle

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itself. Its primary function is to facilitate the further compaction of the "beads-on-a-string" nucleosome array into a more condensed 30 nm chromatin fiber. By stabilizing the entry and exit points of DNA, $H_1$ promotes high-order folding.

**76.** (C) 6
The solenoid model describes the structure of the 30 nm chromatin fiber, where the nucleosome chain is coiled into a left-handed helix. In this model, each complete turn of the solenoid consists of approximately six nucleosomes. This organization provides a six-fold increase in DNA compaction compared to the basic nucleosome filament, playing a critical role in the structural arrangement.

**77.** (A) An abundance of basic amino acids like lysine and arginine
Histones are characterized by an exceptionally high proportion of basic amino acids, particularly lysine and arginine. These residues carry positive charges at physiological pH, allowing them to form strong electrostatic interactions with the negatively charged phosphate groups along the DNA backbone. This chemical affinity is the primary force that drives the tight wrapping of DNA around the histone octamer.

**78.** (B) Naked DNA → Nucleosome → 30 nm fiber → Radial loop domains → Metaphase chromosome
Eukaryotic DNA undergoes several levels of compaction to fit within the nucleus. It begins as naked DNA, which wraps around histones to form nucleosomes. These nucleosomes coil into the 30 nm fiber, which is then organized into radial loop domains anchored to a protein scaffold. During cell division, these loops condense further to form the highly compact metaphase chromosomes.

**79.** (D) Transcriptionally inert, heavily methylated, and hypoacetylated
Heterochromatin represents the highly condensed regions of the eukaryotic genome that are generally transcriptionally inactive. This state is maintained by specific biochemical markers, including heavy DNA methylation and hypoacetylation of histone tails. By remaining tightly packed, heterochromatin sequesters genes from the transcriptional machinery, playing a crucial role in gene silencing and the maintenance of chromosomal structural integrity during division.

**80.** (B) Chromatin relaxation, facilitating transcriptional activation
The acetylation of core histone tails is a key epigenetic modification associated with transcriptional activation. Histone acetyltransferases add acetyl groups to lysine residues, neutralizing their positive charges and weakening the electrostatic attraction between histones and DNA. This results in the relaxation of the chromatin structure, making the DNA more accessible to RNA polymerase and other transcription factors for expression.

**81.** (A) Telomeres and centromeres
Constitutive heterochromatin refers to genomic regions that remain permanently condensed and transcriptionally silent across all cell types and developmental stages. These regions typically consist of highly repetitive DNA sequences and are localized at the centromeres and telomeres of chromosomes. They are essential for proper chromosome segregation during mitosis and provide structural protection to the ends of the linear DNA.

**82.** (C) The unstructured N-terminal tail
Core histone proteins possess unstructured N-terminal tails that extend outward from the nucleosome core particle. These tails are highly susceptible to various post-translational modifications, including acetylation, methylation, and phosphorylation. These modifications function as a molecular code that regulates chromatin structure and gene accessibility by recruiting specific regulatory proteins or altering the physical interactions between nucleosomes in the fiber.

**83.** (D) Contains genes silenced in specific cell types but activatable in others
Facultative heterochromatin consists of genomic regions that can transition between condensed, inactive states and relaxed, active states. Unlike constitutive heterochromatin, which is permanently silent, facultative heterochromatin contains genes that are silenced in specific cell lineages but can be activated in others. This flexibility is essential for cellular differentiation and the regulated expression of developmental genes throughout the life cycle.

**84.** (A) P-3, Q-2, R-4, S-1
Chromatin organization involves different states and structural components. Euchromatin is

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less condensed and transcriptionally active, while constitutive heterochromatin remains permanently packed, especially at centromeres. The nucleosome is the basic unit where DNA wraps around histones. Scaffold proteins are necessary for the high-order arrangement of chromosome loops. Correctly identifying these features is fundamental to understanding how genomes are.

**85.** (D) DnaA binding boxes and AT-rich 13-mer regions
The origin of replication in E. coli, known as oriC, contains specific DNA sequences essential for initiation. It features several DnaA binding boxes, which are recognized by the initiator protein, and a region composed of three highly conserved AT-rich 13-mer sequences. The low number of hydrogen bonds in these AT-rich segments facilitates the local unwinding of DNA during initiation.

**86.** (D) DnaA
DnaA is the primary initiator protein for DNA replication in E. coli. It functions by binding to the specific DnaA boxes within the oriC region. When associated with ATP, DnaA molecules multimerize and cause the adjacent AT-rich 13-mer sequences to melt, creating an open complex. This initial unwinding is a prerequisite for the recruitment of the helicase and replication.

**87.** (D) It acts as a helicase loader, delivering DnaB to the replication fork
During the initiation of DNA replication in E. coli, DnaC acts as a specialized chaperone known as a helicase loader. Its primary role is to facilitate the delivery and loading of the DnaB helicase onto the single-stranded DNA at the origin. DnaC binds to DnaB and stabilizes it in an inactive state until it is correctly positioned at the fork.

**88.** (B) The lagging strand
DNA replication is bidirectional and semi-discontinuous due to the antiparallel nature of the DNA double helix. While the leading strand is synthesized continuously, the lagging strand is synthesized in short, discrete segments called Okazaki fragments. This occurs because DNA polymerase can only add nucleotides in the 5' to 3' direction, necessitating a discontinuous approach as the fork moves.

**89.** (D) Primase (DnaG)
In E. coli, the enzyme primase, also known as DnaG, is responsible for synthesizing short RNA primers. These primers provide the free 3'-OH group necessary for DNA polymerase III to begin synthesis. On the lagging strand, primase must repeatedly synthesize new primers for each Okazaki fragment, ensuring that the replication machinery can continue to duplicate DNA as the fork.

**90.** (A) Both A and R are true and R is the correct explanation of A.
Lagging strand synthesis is inherently discontinuous because DNA polymerases can only extend a DNA chain in the 5' to 3' direction. As the replication fork opens in one direction, the lagging strand template is oriented in the opposite direction. Consequently, the polymerase must synthesize DNA in small segments, moving away from the fork, to accommodate the unwinding.

**91.** (A) DNA Polymerase I
DNA Polymerase I is a multifunctional enzyme that plays a critical role in finishing the synthesis of Okazaki fragments. It utilizes its 5' to 3' exonuclease activity to remove the RNA primers and simultaneously replaces them with DNA nucleotides using its polymerase activity. This process, known as nick translation, ensures that the nascent lagging strand is composed entirely of DNA.

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**92.** (D) Tus protein
The termination of DNA replication in E. coli occurs at specific sequences called Ter sites, which are located opposite the origin. The Tus protein binds to these sites and acts as a directional roadblock, halting the progress of the DnaB helicase and the replication fork. This ensures that the two forks meet correctly and prevents over-replication of the circular.

**93.** (D) To catalyze the formation of a phosphodiester bond between adjacent DNA fragments
DNA ligase is the essential enzyme responsible for sealing the nicks in the sugar-phosphate backbone of the newly synthesized DNA. It catalyzes the formation of a covalent phosphodiester bond between the 3'-OH group of one DNA fragment and the 5'-phosphate group of the adjacent fragment. This action is particularly crucial on

the lagging strand for joining Okazaki fragments together.

**94.** (B) Prevent single-stranded DNA from reannealing or forming secondary structures
Single-strand binding proteins are vital for maintaining the stability of the replication fork. As the DNA double helix is unwound by helicase, SSBs quickly bind to the exposed single strands. This prevents the DNA from reannealing into a double helix or forming internal secondary structures, such as hairpins, which would otherwise impede the progress of the DNA polymerase complex.

**95.** (B) The trombone model
The trombone model describes the coordinated synthesis of the leading and lagging strands by the replisome complex. To allow both strands to be synthesized in the same direction, the lagging strand template is looped back through the polymerase. As synthesis proceeds, this loop grows and then is released, resembling the motion of a trombone slide, ensuring synchronization at the fork.

**96.** (A) I, II and III
Prokaryotic DNA replication is characterized by a semi-conservative and bidirectional process starting from a single origin. The DnaB helicase unwinds DNA in the 5' to 3' direction relative to the lagging strand. To maintain coordination, the lagging strand template is looped. While topoisomerases are involved in termination, the process is primarily dictated by Ter sites and Tus proteins.

**97.** (A) Eukaryotes possess multiple origins per chromosome
A fundamental difference between prokaryotic and eukaryotic DNA replication is the number of origins utilized. Eukaryotic chromosomes are much larger and linear, necessitating the use of multiple origins of replication per chromosome to ensure the entire genome is duplicated within the limited time of the S phase. Prokaryotes, having smaller circular chromosomes, typically rely on a single origin.

**98.** (A) $G_1$ phase
The assembly of the pre-replication complex is a critical regulatory step that occurs during the $G_1$ phase of the eukaryotic cell cycle. This process, known as licensing, involves the recruitment of the origin recognition complex, along with licensing factors like Cdc6 and Cdt1, and the MCM helicase. This ensures that origins are prepared for activation during the subsequent S phase.

**99.** (A) Origin Recognition Complex (ORC)
The Origin Recognition Complex is a highly conserved multiprotein assembly that serves as the foundation for eukaryotic DNA replication initiation. ORC binds specifically to origins of replication across the genome throughout most of the cell cycle. During the $G_1$ phase, it recruits the necessary licensing factors and the MCM helicase to form the pre-replication complex, preparing the DNA.

**100.** (C) Licensing factors such as Cdc6 and Cdt1
To prevent the re-initiation of DNA replication within a single cell cycle, eukaryotic cells strictly regulate licensing factors such as Cdc6 and Cdt1. Once the S phase begins, these proteins are either degraded by the proteasome, exported from the nucleus, or inhibited by specific proteins like geminin. These mechanisms ensure that each origin fires exactly once, maintaining genomic stability.

**101.** (B) Unwinding the DNA double helix ahead of the fork
The MCM complex is the primary replicative helicase in eukaryotic cells. Composed of six different subunits, it is loaded onto the DNA during the $G_1$ phase as part of the pre-replication complex. Once activated during the S phase by specific kinases, the MCM helicase unwinds the DNA double helix, providing the single-stranded templates required for leading and lagging strand synthesis.

**102.** (A) ORC binding → Cdc6/Cdt1 recruitment → MCM loading → Kinase activation (CDK/DDK)
Eukaryotic replication initiation follows a precise sequence of molecular events. It begins with the binding of the Origin Recognition Complex to the DNA, followed by the recruitment of licensing factors Cdc6 and Cdt1. These factors then facilitate the loading of the MCM helicase. Finally, the activation of CDK and DDK kinases during the S phase triggers the start of synthesis.

**103.** (B) The 3' end of the lagging strand template
The end-replication problem refers to the inability of the standard DNA replication machinery to

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completely copy the extreme 3' ends of linear chromosomes. Because DNA polymerase requires an RNA primer to initiate synthesis, the removal of the final primer on the lagging strand leaves a gap that cannot be filled. Without a specialized mechanism, chromosomes would progressively shorten with division.

**104.** (B) Acts as a reverse transcriptase with its own RNA template
Telomerase is a specialized ribonucleoprotein enzyme that addresses the end-replication problem in eukaryotic cells. It functions as a reverse transcriptase, utilizing an internal RNA molecule as a template to synthesize and add repetitive DNA sequences to the 3' ends of chromosomes. By extending the parental DNA strand, telomerase provides the space necessary for primase to initiate lagging strand synthesis.

**105.** (A) 5'-TTAGGG-3'
In human cells, the telomeric regions at the ends of chromosomes consist of thousands of tandem repeats of the hexanucleotide sequence 5'-TTAGGG-3'. These GC-rich repeats are essential for chromosomal stability and protect genetic information from degradation or improper repair. The length of these repeats is maintained by the enzyme telomerase in germ cells, stem cells, and many cancer.

**106.** (C) T-loop
The extreme ends of mammalian chromosomes are organized into a unique structural conformation known as a T-loop. In this structure, the single-stranded 3' overhang of the telomeric DNA invades the double-stranded telomeric region, forming a protective cap. This prevents the chromosome ends from being recognized as double-strand breaks by the cell's DNA damage repair machinery, avoiding catastrophic genomic.

**107.** (B) Progressive telomere shortening with division, leading to senescence
Most normal human somatic cells lack significant telomerase activity, meaning they cannot maintain the length of their telomeres. Consequently, telomeres shorten progressively with each cell division. When telomeres reach a critically short length, the cell enters a state of permanent growth arrest known as senescence. This process acts as a biological clock and serves as a fundamental tumor suppressor.

**108.** (A) P-4, Q-3, R-2, S-1
Many proteins involved in eukaryotic DNA replication have functional equivalents in prokaryotes. The Origin Recognition Complex corresponds to the DnaA initiator protein. The MCM complex functions as the DnaB helicase, while PCNA acts as the sliding clamp, similar to the bacterial beta subunit. Finally, RPA serves as the single-strand binding protein, maintaining the DNA in an accessible state.

**109.** (D) 5' to 3' polymerase activity requiring a primer
All known DNA polymerases share a fundamental catalytic requirement: they can only synthesize DNA in the 5' to 3' direction. Furthermore, they cannot initiate the synthesis of a new DNA strand de novo and must have a pre-existing primer with a free 3'-OH group to add the next nucleotide. This universal property dictates the complex mechanisms of leading and.

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**110.** (C) DNA Polymerase III
DNA Polymerase III is the primary replicative enzyme in E. coli, responsible for the bulk synthesis of both the leading and lagging strands. It is a highly processive and accurate enzyme, functioning as part of a large holoenzyme complex. Its high speed and fidelity are essential for the rapid duplication of the bacterial chromosome during the exponential growth phase of cell.

**111.** (A) 5' to 3' exonuclease activity
E. coli DNA Polymerase I is distinguished by its unique 5' to 3' exonuclease activity, which is located in a distinct domain of the protein. This activity allows the enzyme to degrade DNA or RNA strands ahead of it while simultaneously synthesizing a new DNA strand. This dual function is essential for the removal of RNA primers and the repair of.

**112.** (D) 3' to 5' exonuclease activity of the epsilon ( $\varepsilon$) subunit
The exceptional fidelity of DNA Polymerase III in E. coli is largely due to the proofreading activity of its epsilon subunit. This subunit possesses 3' to 5' exonuclease activity, which allows the enzyme to recognize and remove incorrectly paired nucleotides immediately after they are incorporated. This selF-correcting mechanism significantly reduces the error rate during the process of chromosomal DNA.

**113.** (D) DNA Polymerase alpha ($\alpha$)
In eukaryotic cells, DNA Polymerase alpha plays a critical role in the initiation of DNA synthesis. It exists as a complex with primase activity, allowing it to synthesize a short RNA primer followed by a brief segment of DNA. Once this initial RNA-DNA primer is formed, the replication machinery switches to more processive polymerases to complete the synthesis of the.

**114.** (A) Both A and R are true and R is the correct explanation of A.
DNA Polymerase epsilon is one of the primary replicative polymerases in eukaryotes, specifically tasked with the continuous synthesis of the leading strand. It is characterized by its high processivity and a robust 3' to 5' exonuclease activity, which ensures high fidelity. Its structural organization allows it to remain associated with the replication fork for long distances, facilitating efficient genome.

**115.** (B) DNA Polymerase gamma ($\gamma$)
DNA Polymerase gamma is the specialized enzyme responsible for the replication and repair of the mitochondrial genome in eukaryotic cells. It is the only DNA polymerase found within the mitochondria and is encoded by nuclear genes. Like the primary nuclear replicative polymerases, it possesses both high processivity and proofreading activity to maintain the integrity of the mitochondrial DNA over.

**116.** (D) Beta ($\beta$) clamp
The processivity of the E. coli DNA Polymerase III holoenzyme is drastically increased by the presence of the beta subunit, which functions as a sliding clamp. This ring-shaped protein encircles the DNA and physically tethers the polymerase to the template, preventing it from dissociating. This allows the enzyme to add thousands of nucleotides without falling off, ensuring rapid DNA.

**117.** (A) Primary synthesis of the lagging strand
DNA Polymerase delta is a major eukaryotic DNA polymerase primarily involved in the synthesis of the lagging strand. After DNA Polymerase alpha initiates synthesis, Polymerase delta takes over to extend the Okazaki fragments. It works in conjunction with the PCNA sliding clamp to achieve high processivity and also possesses proofreading activity to ensure the accuracy of the replicated.

**118.** (C) The SOS response to DNA damage
DNA Polymerase II in E. coli is a specialized enzyme that is primarily induced as part of the SOS response to DNA damage. Although it is not the main replicative polymerase, it plays a vital role in DNA repair and can restart replication forks that have stalled due to the presence of lesions in the DNA template, ensuring cellular survival.

**119.** (A) 3' Carbon
All DNA polymerases catalyze the formation of a phosphodiester bond by facilitating a nucleophilic attack. The 3'-hydroxyl group, attached to the 3' carbon of the existing sugar, attacks the alpha-phosphate of the incoming nucleoside triphosphate. This requirement for a free 3'-OH group is why DNA polymerases must have a primer to initiate synthesis and can only grow in direction.

**120.** (A) I, II, IV, V
DNA Polymerase I is a single polypeptide with multiple activities, whereas Polymerase alpha initiates synthesis without proofreading. The Klenow fragment retains polymerase and 3' exonuclease activities but lacks the 5' exonuclease. Processivity measures the efficiency of synthesis before dissociation. PCNA is the eukaryotic sliding clamp. Statements concerning Pol I, Pol alpha, processivity, and PCNA are accurate while the Klenow description.

**121.** (B) Formation of cyclobutane pyrimidine dimers
Exposure to ultraviolet radiation, specifically in the UV-B range, is a major cause of DNA damage in living organisms. The energy from UV light induces the formation of covalent bonds between adjacent pyrimidine bases, such as thymine or cytosine, on the same DNA strand. These cyclobutane pyrimidine dimers distort the double helix and can block replication and transcription if not.

**122.** (D) DNA glycosylases
Base Excision Repair is a critical pathway for fixing small, non-bulky DNA lesions, such as deaminated or oxidized bases. The process is initiated by a class of enzymes called DNA glycosylases, which recognize specific damaged bases. The glycosylase cleaves the N-glycosidic

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bond between the damaged base and the sugar-phosphate backbone, leaving an abasic site that is subsequently repaired by enzymes.

**123.** (D) An AP (apurinic/apyrimidinic) site
The removal of a nitrogenous base by a DNA glycosylase results in the formation of an apurinic or apyrimidinic site, commonly known as an AP site. These sites are unstable and potentially mutagenic if not promptly corrected. In the Base Excision Repair pathway, AP endonucleases recognize these sites and cleave the DNA backbone, initiating the further steps of repair.

**124.** (B) Excises an oligonucleotide patch containing bulky lesions
Nucleotide Excision Repair and Base Excision Repair are distinct pathways for maintaining genomic integrity. While BER targets specific modified bases, NER is responsible for repairing bulky DNA lesions that significantly distort the double helix, such as UV-induced pyrimidine dimers. NER functions by excising a short oligonucleotide fragment containing the damage, which is then replaced by DNA polymerase and sealed by.

**125.** (C) The parental strand is methylated at the adenine of GATC sequences
In E. coli, the mismatch repair system distinguishes the parental DNA strand from the newly synthesized strand by monitoring their methylation status. The parental strand is methylated at the adenine residues of GATC sequences by the Dam methylase. Immediately after replication, the new strand is transiently unmethylated, allowing the repair machinery to identify and correct errors specifically on the nascent DNA.

**126.** (C) MutS
MutS is a key protein in the E. coli mismatch repair pathway, responsible for the initial detection of DNA errors. It scans the double helix and binds specifically to mismatched base pairs or small insertion-deletion loops. Once a mismatch is identified, MutS recruits MutL and MutH to initiate the excision and repair of the incorrect nucleotide on the newly synthesized DNA.

**127.** (B) LexA
The SOS response in E. coli is a global regulatory network that manages severe DNA damage. Under normal physiological conditions, the genes involved in the SOS response are kept in a repressed state by the binding of the LexA protein to their promoter regions. LexA acts as a transcriptional repressor, preventing the expression of repair enzymes until a damage signal is.

**128.** (C) Acts as a co-protease for LexA cleavage
RecA is the primary sensor of DNA damage in E. coli. When it detects single-stranded DNA, which accumulates at stalled replication forks or breaks, it forms a nucleoprotein filament and becomes activated. This active RecA then promotes the selF-cleavage of the LexA repressor. The destruction of LexA results in the induction of the SOS genes, facilitating DNA repair and cell.

**129.** (B) Lack 3' to 5' proofreading and are highly error-prone
Translesion synthesis is an emergency DNA repair mechanism activated during the SOS response. It utilizes specialized DNA polymerases, such as Pol IV and Pol V, which can synthesize DNA across bulky lesions that stall standard polymerases. However, these TLS polymerases lack 3' to 5' proofreading activity and are highly error-prone, often introducing mutations in exchange for allowing replication.

**130.** (D) Non-homologous end joining (NHEJ)
Non-homologous end joining is a primary pathway for repairing double-strand breaks in eukaryotic DNA, especially during the $G_1$ phase when a sister chromatid is unavailable. NHEJ directly ligates the broken ends of the DNA molecule together. While rapid and efficient, this process is inherently error-prone and often results in the small insertion or deletion of nucleotides at the repair.

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**131.** (B) UvrB/UvrC
In the E. coli nucleotide excision repair pathway, the UvrABC complex is responsible for identifying and excising damaged DNA. UvrA and UvrB first recognize the lesion, and then UvrC is recruited to act as an excinuclease. UvrB and UvrC catalyze dual incisions on both the 3' and 5' sides of the damage, allowing a short segment of DNA to be.

**132.** (A) I and II
Deamination of cytosine, not adenine, produces uracil, which is a common source of mutation. Non-homologous end joining is typically associated with eukaryotes and is characterized by its error-prone nature, whereas bacteria primarily use high-fidelity homologous recombination. Nucleotide excision repair is indeed the major system for fixing UV-induced dimers in humans. Therefore, assertions about deamination and NHEJ are incorrect.

**133.** (B) $\alpha_2\beta\beta'\sigma$
The core enzyme of RNA polymerase in E. coli is a multi-subunit complex responsible for the elongation phase of transcription. It is composed of five polypeptide chains: two alpha subunits, one beta subunit, one beta-prime subunit, and one omega subunit. While this core complex can catalyze the synthesis of RNA, it lacks the ability to recognize specific promoter sequences without the.

**134.** (D) Sigma ($\sigma$) factor
The sigma factor is a critical regulatory subunit that associates with the bacterial RNA polymerase core enzyme to form the holoenzyme. Its primary function is to ensure the specific initiation of transcription by recognizing and binding to promoter consensus sequences on the DNA. Once the polymerase has successfully initiated synthesis and moved past the promoter, the sigma factor typically.

**135.** (A) -10 and -35 regions
Prokaryotic promoters are characterized by two highly conserved consensus sequences located upstream of the transcription start site. These are the -10 region, also known as the Pribnow box, and the -35 region. The -35 element is primarily involved in the initial binding of the RNA polymerase holoenzyme, while the -10 element is essential for the subsequent unwinding of DNA.

**136.** (D) Pribnow box
The -10 consensus sequence in bacterial promoters is frequently referred to as the Pribnow box. It typically consists of the sequence TATAAT and is located approximately ten nucleotides upstream of the transcription start site. Due to its high adenine and thymine content, this region is easily unwound, facilitating the transition from a closed to an open promoter complex during transcription.

**137.** (A) Sigma 32 ($\sigma^{32}$)
In E. coli, the heat shock response is regulated by the alternative sigma factor known as Sigma 32. When the temperature increases, Sigma 32 levels rise and it directs RNA polymerase to transcribe a specific set of genes encoding heat shock proteins and chaperones. These proteins help the cell cope with protein misfolding and other stresses caused by the elevated temperature.

**138.** (C) Melting of DNA around the -10 element
The transition from a closed promoter complex to an open promoter complex is a vital step in the initiation of prokaryotic transcription. This process involves the localized melting, or unwinding, of approximately 12 to 14 base pairs of DNA centered around the -10 consensus sequence. This unwinding provides the single-stranded template necessary for the RNA polymerase to begin RNA.

**139.** (D) Synthesis and release of short RNAs (2-10 nt) during initiation
Abortive initiation is a characteristic phenomenon that occurs during the early stages of transcription initiation in bacteria. During this process, the RNA polymerase holoenzyme synthesizes and releases several short RNA transcripts, typically two to ten nucleotides in length, without leaving the promoter. This cycle continues until the polymerase undergoes a conformational change that allows it to escape the promoter and.

**140.** (A) GC-rich hairpin followed by a run of uracil residues
Rho-independent, or intrinsic, termination of transcription in bacteria is determined by specific sequences at the end of a gene. These sequences typically encode a GC-rich region followed by a series of uracil residues in the nascent RNA. The GC-rich segment forms a stable hairpin structure that causes the RNA polymerase to stall, while the weak A-U base pairing facilitates.

**141.** (D) Binds to a rut site and acts as an RNA-DNA helicase
Rho-dependent termination involves a specialized protein called Rho, which functions as an ATP-dependent RNA-DNA helicase. Rho binds to a specific C-rich sequence on

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the nascent RNA transcript known as the rut site. It then moves along the RNA toward the transcribing polymerase. Upon catching the stalled polymerase, Rho uses its helicase activity to unwind the RNA-DNA hybrid, releasing the.

**142.** (A) A purine (A or G)
In the majority of prokaryotic promoters, the transcription start site, designated as the +1 position, is a purine nucleotide, either adenine or guanine. This preference is consistent across many different genes and species. The selection of a purine at the start site is thought to facilitate the efficient initiation of RNA synthesis by providing a stable initial nucleotide for polymerase.

**143.** (D) The RNA exit channel
During the elongation phase of transcription, the nascent RNA transcript is synthesized within the catalytic cleft of the RNA polymerase. As the chain grows, it is directed out of the enzyme through a specialized structural feature known as the RNA exit channel. This ensures that the newly formed RNA does not interfere with the template DNA or the incoming nucleotides during.

**144.** (C) Rifampicin
Rifampicin is a potent antibiotic that selectively inhibits bacterial transcription by binding to the beta subunit of RNA polymerase. This binding physically blocks the path of the nascent RNA transcript, effectively preventing the initiation of RNA synthesis and the transition to the elongation phase. Because it does not affect eukaryotic RNA polymerases, rifampicin is an important drug for treating infections.

**145.** (B) RNA Polymerase II
RNA Polymerase II is the primary enzyme responsible for transcribing all protein-coding genes in eukaryotic cells. It synthesizes pre-messenger RNA, which is later processed into mature mRNA for translation. Additionally, RNA Polymerase II is involved in the production of several small nuclear RNAs and microRNAs. Its activity is highly regulated by a complex array of transcription factors and enhancer.

**146.** (C) The large 45S ribosomal RNA precursor
Eukaryotic RNA Polymerase I is a specialized enzyme located within the nucleolus, the site of ribosome biogenesis. Its sole function is to transcribe the large ribosomal RNA precursor, known as 45S pre-rRNA in humans. This single transcript is later processed into the 18S, 5.8S, and 28S ribosomal RNAs, which are essential structural and catalytic components of the eukaryotic ribosomal.

**147.** (B) The TATA box
Many eukaryotic core promoters transcribed by RNA Polymerase II contain a highly conserved sequence known as the TATA box. Typically located 25 to 30 base pairs upstream of the transcription start site, this AT-rich element is recognized by the TATA-binding protein. Its presence is crucial for the correct positioning of the polymerase and the efficient assembly of the pre-initiation.

**148.** (C) TFIID
TFIID is a foundational general transcription factor required for the initiation of eukaryotic transcription by RNA Polymerase II. It is a large multiprotein complex that includes the TATA-binding protein and several TBP-associated factors. TFIID recognizes and binds to the TATA box and other promoter elements, providing a scaffold for the recruitment of other transcription factors and the polymerase enzyme.

**149.** (A) TFIID
The TATA-binding protein is a vital subunit of the TFIID complex and plays a central role in eukaryotic transcription initiation. TBP specifically recognizes and binds to the minor groove of the TATA box, causing a dramatic bend in the DNA. This structural change facilitates the recruitment of other general transcription factors, such as TFIIB, ensuring the accurate positioning of polymerase.

**150.** (C) DNA helicase activity and CTD kinase activity
TFIIH is a complex general transcription factor with multiple essential enzymatic activities. During the initiation of eukaryotic transcription, its helicase subunits utilize ATP to unwind the DNA at the promoter, forming the open complex. Additionally, its kinase subunit phosphorylates the C-terminal domain of RNA Polymerase II, a critical modification that triggers promoter clearance and the transition to elongation.

**151.** (D) The C-terminal domain (CTD) of Rpb1
The C-terminal domain of the largest subunit of RNA Polymerase II, Rpb1, is a unique regulatory

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region composed of multiple heptapeptide repeats. Extensive phosphorylation of the serine residues within these repeats is required for the polymerase to escape the promoter and enter the elongation phase. This modification also serves to recruit various RNA processing enzymes, including those for capping and.

**152.** (A) Position- and orientation-independent function
Enhancers are powerful cis-acting regulatory elements that significantly increase the transcription of target genes in eukaryotes. They are characterized by their ability to function independently of their orientation and their distance from the promoter, often located thousands of base pairs away. Enhancers provide binding sites for specific activator proteins that interact with the basal transcription machinery to modulate gene levels.

**153.** (D) DNA looping to bring activator proteins into spatial proximity
Enhancer elements can regulate genes located at great distances by utilizing a mechanism known as DNA looping. Activator proteins bound to the enhancer interact with the mediator complex and general transcription factors at the promoter, causing the intervening DNA to loop out. This physical proximity allows the enhancer to effectively recruit and stabilize the transcription machinery, thereby increasing the target.

**154.** (C) tRNAs, 5S rRNA, and small non-coding RNAs
RNA Polymerase III is responsible for the transcription of a diverse set of small, non-coding RNA molecules in eukaryotic cells. Its primary targets include all transfer RNA genes and the 5S ribosomal RNA gene. Additionally, it synthesizes the $U_6$ small nuclear RNA, which is involved in splicing, and several other small regulatory RNAs essential for various aspects of cellular.

**155.** (B) RNA Polymerase II
Alpha-amanitin is a highly toxic cyclic peptide produced by certain mushrooms that acts as a potent inhibitor of eukaryotic transcription. It functions by binding to the back of RNA Polymerase II, blocking its translocation along the DNA template. RNA Polymerase II is extremely sensitive to this toxin, while RNA Polymerase III is moderately sensitive and RNA Polymerase I is resistant.

**156.** (B) III only
Enhancers are versatile elements that function regardless of their position relative to the gene. Basal transcription factors are essential for initiating transcription at the promoter. While RNA Polymerase I transcribes most ribosomal RNAs, the 5S rRNA is uniquely synthesized by RNA Polymerase III. The mediator complex is a key coactivator that bridges regulatory proteins. Therefore, the statement regarding Pol.

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**157.** (A) A 7-methylguanosine cap
The maturation of eukaryotic pre-mRNA begins during transcription with the addition of a 7-methylguanosine cap to the 5' end. This co-transcriptional modification occurs when the nascent transcript is only about 20 to 30 nucleotides long. The cap is essential for protecting the mRNA from degradation by exonucleases and plays a vital role in the export and subsequent translation of mRNA.

**158.** (C) A 5'-to-5' triphosphate bridge
The 7-methylguanosine cap is attached to the 5' end of the eukaryotic pre-mRNA through a unique chemical linkage. Unlike the standard 5' to 3' phosphodiester bonds found in the rest of the RNA molecule, the cap is joined via a 5'-to-5' triphosphate bridge. This unusual orientation protects the transcript from 5' exonucleases, which cannot recognize or cleave this bond.

**159.** (A) Protects mRNA from 5' exonucleases and assists in ribosome binding
The 5' cap provides several critical functional advantages to the eukaryotic mRNA molecule. It serves as a protective barrier against 5' exonucleases, significantly increasing the transcript's stability in the cytoplasm. Furthermore, the cap is recognized by the translation initiation machinery, facilitating the binding of the ribosome and ensuring that the mRNA is correctly positioned for the start of protein.

**160.** (B) Post-transcriptional addition by poly(A) polymerase
The poly(A) tail at the 3' end of eukaryotic mRNA is added post-transcriptionally through a two-step process. First, the nascent pre-mRNA is cleaved at a specific site, and then

the enzyme poly(A) polymerase adds a string of approximately 200 adenine nucleotides without a DNA template. This tail is essential for mRNA stability, nuclear export, and efficient translation by ribosomal.

**161.** (A) AAUAAA
The 3' processing of eukaryotic pre-mRNA is directed by a highly conserved signal sequence, typically AAUAAA, located upstream of the cleavage site. This sequence is recognized by specific proteins that recruit the cleavage factors and poly(A) polymerase to the transcript. Cleavage usually occurs 10 to 30 nucleotides downstream of this signal, followed by the immediate addition of poly(A).

**162.** (D) Introns
Splicing is a fundamental process in eukaryotic gene expression where non-coding sequences called introns are removed from the pre-messenger RNA transcript. The remaining coding segments, known as exons, are then precisely ligated together to form a continuous sequence. This maturation step is essential for the production of functional mRNA that can be correctly translated into a protein by ribosomes.

**163.** (D) snRNPs such as $U_1$, $U_2$, $U_4$, $U_5$, and $U_6$
The spliceosome is a large macromolecular complex that catalyzes the removal of introns from eukaryotic pre-mRNA. It is composed of five specialized small nuclear ribonucleoprotein particles, designated as $U_1$, $U_2$, $U_4$, $U_5$, and $U_6$. These snRNPs utilize their RNA components to recognize specific sequences at the splice sites and coordinate the transesterification reactions required to excise.

**164.** (A) Adenine in the intronic branch point sequence
The first step of nuclear pre-mRNA splicing is initiated by a nucleophilic attack on the 5' splice site. This reaction is carried out by the 2'-hydroxyl group of a specific adenine residue located within the intronic branch point sequence. This conserved adenine is essential for the formation of the lariat intermediate, which is a hallmark of the spliceosome-mediated splicing.

**165.** (A) A lariat structure
During the process of splicing, the excision of an intron proceeds through the formation of a characteristic intermediate structure known as a lariat. This occurs when the 5' end of the intron is covalently linked to the 2'-OH group of the branch point adenine. The resulting loop structure remains attached to the 3' exon until the second transesterification reaction.

**166.** (D) Produce multiple protein isoforms from a single locus
Alternative splicing is a key regulatory mechanism that allows eukaryotic cells to produce multiple distinct protein isoforms from a single gene. By selectively including or excluding different exons during the splicing process, the cell can generate proteins with varying functional properties or cellular localizations. This process significantly increases the diversity and complexity of the proteome without requiring a larger.

**167.** (C) Entirely selF-splicing ribozymes
Group I and Group II introns are unique because they are entirely selF-splicing ribozymes, meaning they can catalyze their own excision from an RNA transcript without the need for the spliceosome or other protein enzymes. These introns use complex three-dimensional folding and specific chemical reactions to remove themselves, demonstrating the remarkable catalytic potential of RNA molecules in biological contexts.

**168.** (A) $U_1$
The $U_1$ small nuclear ribonucleoprotein particle plays a critical role in the early stages of spliceosome assembly. Its RNA component contains a sequence that is perfectly complementary to the conserved 5' splice site of the pre-mRNA. By base-pairing with this site, $U_1$ snRNP correctly identifies the start of the intron, initiating the recruitment of other factors needed for.

**169.** (B) 61
The genetic code consists of 64 possible three-nucleotide combinations, known as codons. Out of these, 61 are classified as sense codons because they specify a particular amino acid for incorporation into a protein. The remaining three codons serve as termination signals, which do not encode any amino acid and instead instruct the ribosome to stop translation and release the.

**170.** (D) Degeneracy (Redundancy)
The genetic code is characterized by degeneracy, or redundancy, meaning that most amino acids are specified by more than one codon. This

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feature provides a buffer against mutations, as a single nucleotide change in the third position of a codon often results in the same amino acid being incorporated. Degeneracy allows for flexibility in the DNA sequence while maintaining protein.

**171.** (A) AUG
The codon AUG serves as the universal start signal for protein synthesis in both prokaryotes and eukaryotes. It establishes the correct reading frame for the messenger RNA and encodes the amino acid methionine. In bacteria, a specialized form called N-formylmethionine is used at this initial position. The precise identification of the AUG codon is essential for accurate translation.

**172.** (A) UAA, UAG, UGA
The termination of protein synthesis is signaled by three specific codons: UAA, UAG, and UGA. These are known as stop codons or nonsense codons, as they do not specify an amino acid. When the ribosome encounters one of these sequences in the A-site, it recruits release factors that trigger the hydrolysis of the finished polypeptide chain from the.

**173.** (A) Base-pairing relaxation at the third codon position
The wobble hypothesis, proposed by Francis Crick, explains how a single transfer RNA can recognize multiple codons. It suggests that while the first two positions of the codon follow strict Watson-Crick base-pairing, the third position, or wobble position, has more flexibility. This allows for non-canonical base pairing between the tRNA anticodon and the mRNA codon, reducing the required.

**174.** (A) Uracil, Cytosine, or Adenine
Inosine is a modified nitrogenous base frequently found at the first position of the tRNA anticodon. Due to its unique structure, inosine can form hydrogen bonds with three different bases at the third position of the mRNA codon: uracil, cytosine, and adenine. This versatility allows a single tRNA containing inosine to recognize and translate multiple synonymous codons for.

**175.** (B) Unequal frequency of synonymous codon usage
Codon usage bias refers to the non-random frequency with which synonymous codons are used to encode the same amino acid in a given organism. While multiple codons may specify the same amino acid, certain species preferentially use specific codons over others. This bias often correlates with the abundance of corresponding transfer RNAs and can significantly influence the efficiency and.

**176.** (C) Strict non-overlapping and continuous reading
A fundamental rule of the genetic code is its non-overlapping and continuous nature. Once translation begins at a specific start codon, the ribosome reads the mRNA sequence three nucleotides at a time without skipping any bases. This lack of punctuation ensures that the correct reading frame is maintained throughout the entire gene, resulting in the production of a specific.

**177.** (A) Tryptophan
Although the genetic code is nearly universal, several exceptions exist, particularly in mitochondrial genomes. In human mitochondria, the codon UGA, which serves as a stop signal in the standard nuclear code, is reassigned to encode the amino acid tryptophan. This variation reflects the evolutionary divergence of mitochondria and highlights the specialized nature of their independent translation machinery within cells.

**178.** (B) 31
Due to the wobble mechanism, an organism does not need 61 different transfer RNAs to translate all the sense codons. The wobble hypothesis predicts that a minimum of 31 distinct tRNAs is sufficient to recognize all the codons that specify amino acids. This efficiency allows cells to maintain a smaller set of tRNA genes while still accurately decoding genomes.

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**179.** (A) Nonsense mutation
A nonsense mutation is a type of point mutation that changes a sense codon specifying an amino acid into a premature stop codon. For example, changing the tyrosine codon UAU to the stop codon UAA will cause the ribosome to terminate translation early. This usually results in a truncated and non-functional protein, which can have significant phenotypic consequences for.

**180.** (A) I, IV, V
The genetic code is both degenerate and unambiguous, ensuring precise translation. While it is mostly universal, rare reassignments like selenocysteine occur at specific UGA

sites. The reading frame is established at the start codon, not the poly-A tail. Furthermore, wobble base pairing involves the 5' base of the anticodon. Therefore, statements on degeneracy, selenocysteine, and continuity are.

**181.** (B) 50S and 30S
The prokaryotic ribosome is a large molecular machine with a total sedimentation coefficient of 70S. It is composed of two distinct subunits: the large 50S subunit and the small 30S subunit. Together, these subunits coordinate the complex interactions between messenger RNA and transfer RNA required for protein synthesis. The specific composition of these subunits is essential for their performance.

**182.** (D) 16S rRNA
The 16S ribosomal RNA is a fundamental component of the small 30S subunit in prokaryotic ribosomes. It plays a critical role in the initiation of translation by base-pairing with the Shine-Dalgarno sequence on the messenger RNA, ensuring the correct positioning of the start codon. Additionally, the 16S rRNA provides the structural framework for the assembly of the small.

**183.** (B) 80S
Eukaryotic ribosomes are larger and more complex than their prokaryotic counterparts, with an intact sedimentation coefficient of 80S. They are composed of a large 60S subunit and a small 40S subunit. These ribosomes are responsible for all protein synthesis in the cytoplasm and on the rough endoplasmic reticulum, reflecting the increased regulatory requirements of eukaryotic cell biology and.

**184.** (D) The large subunit rRNA (e.g., 23S)
The peptidyl transferase center, which catalyzes the formation of peptide bonds during protein synthesis, is located within the large ribosomal subunit. In prokaryotes, this catalytic activity is performed by the 23S ribosomal RNA rather than a protein. This makes the ribosome a ribozyme, highlighting the essential role of RNA in the fundamental processes of life and evolutionary.

**185.** (B) Attaching the correct amino acid to its cognate tRNA
Aminoacyl-tRNA synthetases are essential enzymes that ensure the accuracy of the genetic code by attaching the correct amino acid to its cognate transfer RNA. This process, known as charging or aminoacylation, involves high specificity for both the amino acid and the tRNA molecule. These enzymes effectively act as the molecular translators that link the nucleotide sequence to protein.

**186.** (B) ATP
The process of charging a transfer RNA with its specific amino acid is an endergonic reaction that requires an input of energy. This energy is provided by the hydrolysis of ATP. The aminoacyl-tRNA synthetase enzyme utilizes the high-energy phosphate bond to activate the amino acid, creating a reactive intermediate that can then be covalently linked to the tRNA.

**187.** (D) Aminoacyl-AMP (aminoacyl-adenylate)
The charging of a transfer RNA occurs in a two-step reaction catalyzed by aminoacyl-tRNA synthetase. In the first step, the enzyme activates the amino acid by reacting it with ATP to form a high-energy aminoacyl-adenylate intermediate, also known as aminoacyl-AMP. In the second step, this activated amino acid is transferred to the 3' hydroxyl group of the tRNA.

**188.** (B) Hydrolyzing the ester bond of mischarged tRNAs
To maintain high translational fidelity, many aminoacyl-tRNA synthetases possess a specialized editing, or proofreading, mechanism. If the enzyme mistakenly attaches the wrong amino acid to a tRNA, the editing site recognizes the mismatch and hydrolyzes the ester bond, releasing the incorrect amino acid. This quality control step ensures that only correctly charged tRNAs are delivered for protein synthesis.

**189.** (B) E site → P site → A site
A functional ribosome contains three distinct binding sites for transfer RNA, arranged in a specific spatial sequence. In the direction of translation, these are the E site, the P site, and the A site. The A site receives the incoming aminoacyl-tRNA, the P site holds the tRNA with the growing polypeptide chain, and the E site facilitates the exit.

**190.** (B) P site
During the elongation phase of protein synthesis, the growing polypeptide chain is physically anchored to a transfer RNA molecule located in the P site of the ribosome. As each new

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amino acid is added at the A site, the chain is transferred to the new tRNA, and the ribosome translocates, moving the peptidyl-tRNA back into the P.

**191.** (A) 28S, 5.8S, and 5S
The large 60S ribosomal subunit in eukaryotes is a complex assembly containing three different ribosomal RNA molecules. These are the 28S, 5.8S, and 5S rRNAs. Together with numerous ribosomal proteins, these RNA molecules provide the structural integrity and catalytic activity necessary for peptide bond formation. The presence of these multiple RNA types reflects the complexity of eukaryotic ribosomes.

**192.** (C) rRNA forms the catalytic core, while proteins provide stability
The relationship between the RNA and protein components of the ribosome is fundamental to its function. Research has shown that the ribosomal RNA forms the catalytic core of the complex, performing the essential role of peptidyl transferase activity. The ribosomal proteins, while numerous, primarily serve to stabilize the three-dimensional structure of the rRNA and assist in the assembly process.

**193.** (A) On mRNA, 5 to 10 nucleotides upstream of AUG
In prokaryotes, the initiation of translation requires the precise identification of the start codon on the messenger RNA. This is achieved through the Shine-Dalgarno sequence, a purine-rich region located approximately five to ten nucleotides upstream of the AUG codon. This sequence is recognized by the small ribosomal subunit, ensuring that the ribosome is correctly positioned to begin.

**194.** (A) The 3' end of the 16S rRNA
The Shine-Dalgarno sequence on the bacterial messenger RNA functions by base-pairing with a complementary sequence, known as the anti-Shine-Dalgarno sequence, located at the 3' end of the 16S ribosomal RNA. This RNA-RNA interaction is essential for the stable binding of the 30S ribosomal subunit to the mRNA and the accurate alignment of the start codon in the P.

**195.** (B) N-formylmethionine (fMet)
In E. coli and other bacteria, the initiation of protein synthesis utilizes a specialized initiator transfer RNA that carries N-formylmethionine. This modified amino acid is the first residue incorporated into every newly synthesized bacterial polypeptide. The formyl group helps distinguish the initiator tRNA from the standard methionyl-tRNA used during elongation, ensuring that translation only begins at the correct codon.

**196.** (C) IF3
Initiation factor IF3 is a critical regulatory protein in prokaryotic translation. Its primary function is to bind to the small 30S ribosomal subunit and physically prevent it from prematurely associating with the large 50S subunit. This ensures that the 30S subunit remains free to bind to the messenger RNA and the initiator tRNA, forming the correct pre-initiation complex.

**197.** (D) Kozak sequence
Eukaryotic translation initiation follows a scanning mechanism where the 40S ribosomal subunit binds to the 5' cap and moves along the mRNA to find the start codon. The efficiency of this process is influenced by the surrounding sequence, known as the Kozak sequence. A start codon embedded within a strong Kozak consensus is more likely to be recognized by ribosomes.

**198.** (A) EF-Tu (prokaryotes) / $eEF_1A$ (eukaryotes)
During the elongation cycle of protein synthesis, the delivery of the correct aminoacyl-tRNA to the ribosomal A-site is facilitated by specialized elongation factors. In prokaryotes, this factor is EF-Tu, while the eukaryotic equivalent is $eEF_1A$. These factors bind the charged tRNA and GTP, ensuring that only the tRNA with the correct anticodon is accommodated in the ribosome.

**199.** (D) Peptide bond formation via nucleophilic attack
Once the correct aminoacyl-tRNA is successfully positioned in the A-site of the ribosome, the process of peptide bond formation occurs immediately. This reaction is catalyzed by the peptidyl transferase center of the large ribosomal subunit. The amino group of the amino acid in the A-site performs a nucleophilic attack on the ester bond of the peptidyl-tRNA in the P site.

**200.** (C) EF-G
Following the formation of a peptide bond, the ribosome must move exactly three nucleotides along the messenger RNA to the next codon.

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This process, known as translocation, requires energy from GTP hydrolysis and is mediated by specific elongation factors: EF-G in prokaryotes and $eEF_2$ in eukaryotes. Translocation shifts the deacylated tRNA to the E site and the peptidyl-tRNA to P.

**201.** (A) Protein release factors (RFs) mimicking tRNA
The termination of translation is triggered when a stop codon enters the A-site of the ribosome. Since there are no transfer RNAs for these codons, the ribosome instead recruits protein release factors. These factors chemically and structurally mimic a tRNA molecule, entering the A-site and stimulating the peptidyl transferase center to hydrolyze the bond between the finished polypeptide and tRNA.

**202.** (A) UAA and UAG
In E. coli, the termination of protein synthesis involves multiple release factors with distinct codon specificities. Release Factor 1 is responsible for recognizing and binding to the stop codons UAA and UAG. A different factor, $RF_2$, recognizes UAA and UGA. By ensuring that all three termination signals are efficiently detected, these factors facilitate the timely release of newly synthesized proteins.

**203.** (A) Transesterification forming an amide bond
The peptidyl transferase reaction catalyzed by the ribosome is chemically a transesterification that results in the formation of an amide bond. In this reaction, the amino group of the aminoacyl-tRNA in the A-site attacks the ester linkage between the growing polypeptide chain and the tRNA in the P-site. This transfer effectively extends the polypeptide chain by one amino acid residue during.

**204.** (B) Tetracycline
Tetracycline is a broad-spectrum antibiotic that inhibits bacterial protein synthesis by targeting the small 30S ribosomal subunit. It binds reversibly to the A-site, physically blocking the access of aminoacyl-tRNA molecules. By preventing the incorporation of new amino acids into the growing polypeptide chain, tetracycline effectively halts bacterial growth. Because it does not bind to eukaryotic ribosomes, it is selective.

**205.** (D) Repressed by LacI binding to the operator
The E. coli lactose operon is a classic example of a negatively regulated inducible system. In the default state, which occurs in the absence of lactose, the LacI repressor protein binds tightly to the operator sequence. This binding physically blocks RNA polymerase from transcribing the structural genes, ensuring that the cell does not waste energy producing enzymes for an absent substrate.

**206.** (C) Allolactose
Allolactose is the natural inducer of the lac operon in E. coli. It is an isomer of lactose produced at low levels by the enzyme beta-galactosidase. When lactose enters the cell, allolactose is formed and binds to the LacI repressor, causing a conformational change that reduces its affinity for the operator. This induction allows RNA polymerase to begin transcribing operon genes.

**207.** (A) lacZ
The lacZ gene is a critical component of the lactose operon, encoding the enzyme beta-galactosidase. This enzyme performs two vital functions: it hydrolyzes lactose into the simple sugars glucose and galactose, and it converts lactose into the inducer allolactose. The expression of lacZ is essential for the bacterial cell to utilize lactose as a carbon and energy source during growth.

**208.** (B) Cyclic AMP (cAMP)
The lac operon is subject to positive control through catabolite repression, which responds to the availability of glucose. When glucose levels are low, the concentration of the small effector molecule cyclic AMP increases in the cell. cAMP then binds to the Catabolite Activator Protein, forming a complex that enhances the transcription of the operon by recruiting RNA polymerase to promoter.

**209.** (C) Low glucose, high lactose
Transcription of the lac operon reaches its maximal level only when two environmental conditions are met simultaneously. First, lactose must be present to produce allolactose and remove the repressor. Second, glucose levels must be low to ensure high concentrations of cAMP, which activates the CAP protein. This dual requirement ensures that the operon is only fully active when absolutely needed.

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**210.** (D) Recruits and stabilizes RNA polymerase at the promoter
When cAMP-CAP complex binds to its specific site upstream of the lac promoter, it facilitates transcription by directly interacting with RNA polymerase. This binding helps recruit the polymerase to the promoter and stabilizes the formation of the closed complex. By enhancing the enzyme's affinity for the DNA, the cAMP-CAP complex ensures high levels of gene expression in the absence of glucose.

**211.** (A) Tryptophan acts as a co-repressor for the Trp repressor
The E. coli tryptophan operon is a negatively regulated repressible system. Under conditions where exogenous tryptophan is abundant, the amino acid acts as a co-repressor. Tryptophan binds to the inactive Trp repressor protein, causing a conformational change that allows it to bind to the operator. This blocks RNA polymerase and prevents the unnecessary synthesis of enzymes for tryptophan production in.

**212.** (B) Attenuation
Attenuation is a secondary regulatory mechanism used by the trp operon to fine-tune gene expression based on the availability of tryptophan. It relies on a specialized leader sequence that contains a small open reading frame with consecutive tryptophan codons. Depending on the concentration of charged tRNA-Trp, the leader transcript folds into structures that either terminate or allow transcription completion.

**213.** (D) Transcription and translation
The mechanism of attenuation in prokaryotic operons is fundamentally dependent on the tight coupling of transcription and translation. Because bacteria lack a nuclear envelope, ribosomes can begin translating the leader RNA while it is still being synthesized by RNA polymerase. This allows the ribosome's progress to directly influence the folding of the nascent RNA, determining if polymerase will continue.

**214.** (A) Both A and R are true and R is the correct explanation of A.
During the attenuation of the trp operon under low tryptophan conditions, the ribosome stalls at the consecutive tryptophan codons in the leader sequence. This stalling physically prevents the formation of the terminator hairpin. Instead, the nascent RNA folds into an alternative anti-terminator structure, which allows RNA polymerase to continue transcribing the structural genes, ensuring selF synthesis of tryptophan.

**215.** (B) Constitutive expression in the absence of lactose
A mutation that completely inactivates the lacI gene results in a constitutive phenotype for the lactose operon. In the absence of a functional repressor protein, nothing prevents RNA polymerase from binding to the promoter and transcribing the structural genes. Consequently, the enzymes for lactose metabolism are produced continuously, even when lactose is not present in the environment, leading to energy.

**216.** (B) I, III, IV
The lac repressor functions by physically blocking RNA polymerase at the operator. Attenuation is a complex mechanism observed in both repressible and some inducible operons. Glucose levels and cAMP concentrations are inversely related, influencing CAP activity. The trp leader uniquely contains two consecutive tryptophan codons to sense tRNA availability. Therefore, statements on the repressor, cAMP, and trp codons are accurate.

**217.** (A) Transition
A transition is a specific type of point mutation that involves the substitution of one purine for another purine, or one pyrimidine for another pyrimidine. For example, replacing adenine with guanine is a transition. These mutations are generally more common than transversions because they involve chemically similar bases, making them more likely to occur spontaneously during DNA replication.

**218.** (D) Guanine to Cytosine
A transversion is a point mutation that involves the substitution of a purine base for a pyrimidine base, or vice versa. An example of this is the replacement of guanine, a purine, with cytosine, a pyrimidine. Transversions cause a more significant distortion of the DNA double helix than transitions and are less common due to the chemical differences between bases.

**219.** (A) Thymine
The deamination of 5-methylcytosine is a frequent source of spontaneous mutations in

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eukaryotic genomes. When this modified base loses its amino group, it is converted directly into thymine. Since thymine is a natural component of DNA, the mismatch repair machinery may fail to recognize the error, resulting in a permanent C-to-T transition mutation. These sites are genomic hotspots.

**220.** (D) Insertions or deletions leading to frameshifts
Intercalating agents, such as ethidium bromide, are flat molecules that slide between the stacked nitrogenous bases of the DNA double helix. During replication, their presence causes the DNA polymerase to stutter, leading to the insertion or deletion of one or more nucleotides. This typically results in frameshift mutations, which can drastically alter the coding sequence and function of proteins.

**221.** (B) Frameshift mutation altering all downstream amino acids
An insertion of a single nucleotide into the coding sequence of a gene causes a frameshift mutation. Because the genetic code is read in non-overlapping triplets, this addition shifts the entire reading frame for all subsequent codons. This usually results in a completely different amino acid sequence and often leads to a premature stop codon, producing a non-functional truncated protein.

**222.** (C) Guanine
5-bromouracil is a base analog that is chemically similar to thymine. While it usually pairs with adenine, it frequently undergoes tautomeric shifts into a rare enol form. In this state, 5-bromouracil incorrectly base-pairs with guanine during DNA replication. This leads to a permanent transition mutation from an A-T pair to a G-C pair in subsequent generations of.

**223.** (B) Production of a truncated, non-functional polypeptide
A nonsense mutation is a point mutation that creates a premature stop codon in the messenger RNA. When this occurs early in the coding sequence of a gene, the ribosome terminates translation before the full protein is synthesized. The resulting truncated polypeptide is typically non-functional and is often rapidly degraded by the cell, leading to a complete loss of function.

**224.** (A) Gamma rays and X-rays
Ionizing radiation, including X-rays and gamma rays, possesses high energy that can directly break the chemical bonds in DNA. This often results in widespread double-strand breaks, which are highly lethal to the cell if not repaired. Furthermore, ionizing radiation can generate reactive oxygen species that cause oxidative damage to the nitrogenous bases, leading to various types of genomic.

**225.** (A) Cytosine
Tautomeric shifts are spontaneous rearrangements of the hydrogen atoms in nitrogenous bases. For example, adenine normally exists in the amino form but can briefly shift into a rare imino form. In this state, adenine forms stable hydrogen bonds with cytosine instead of thymine. If replication occurs during this shift, a permanent transition mutation will be incorporated into the DNA molecule.

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**226.** (A) Deaminates cytosine to uracil and adenine to hypoxanthine
Nitrous acid is a potent chemical mutagen that functions by deaminating nitrogenous bases. It converts cytosine into uracil and adenine into hypoxanthine. Since uracil pairs with adenine and hypoxanthine pairs with cytosine, these changes result in permanent transition mutations during the next round of DNA replication. Nitrous acid effectively alters the genetic code without breaking the sugar-phosphate DNA.

**227.** (A) Conservative missense mutation
A conservative missense mutation is a type of point mutation that replaces one amino acid with another that has similar chemical properties. For instance, substituting leucine with isoleucine, both of which are nonpolar, often has minimal impact on the protein's overall folding and biological activity. These mutations are frequently neutral and may persist in a population without negative natural selection.

**228.** (A) Gamma rays and X-rays
High-energy radiation, such as gamma rays and X-rays, is highly penetrating and can cause significant damage to the genetic material. By knocking electrons out of atoms, this ionizing radiation generates free radicals that attack the DNA backbone, resulting in complex lesions and double-strand breaks. These breaks are difficult

to repair accurately and often lead to large-scale chromosomal rearrangements.

**229.** (A) Uniparental maternal inheritance
Extrachromosomal inheritance, also known as cytoplasmic inheritance, typically displays a pattern of uniparental maternal transmission in animals. This occurs because the egg cell provides almost all the cytoplasm and organelles, including mitochondria, to the zygote, while the sperm contributes primarily the nuclear genome. Consequently, traits encoded by these organelle genomes are passed from the mother to all her children.

**230.** (C) Small, circular, double-stranded DNA (16.5 kb)
The human mitochondrial genome is a small, circular, double-stranded DNA molecule approximately 16.5 kilobases in length. Unlike the nuclear genome, mtDNA is highly compact, containing very few non-coding sequences and lacking histones. Each mitochondrion contains multiple copies of this genome, which encodes several essential proteins for oxidative phosphorylation as well as the specialized RNAs for their independent.

**231.** (B) Autonomous, selF$^-$replicating extrachromosomal DNA
Bacterial plasmids are defined as small, circular, double-stranded DNA molecules that exist independently of the primary bacterial chromosome. They are autonomous and selF$^-$ replicating extrachromosomal elements that often carry genes providing selective advantages, such as antibiotic resistance or metabolic capabilities. Plasmids are easily transferred between bacteria through horizontal gene transfer, playing a significant role in bacterial evolution and fitness.

**232.** (B) Inability of related plasmids to coexist in one cell line
Plasmid incompatibility is a biological phenomenon where two related plasmids cannot be stably maintained within the same bacterial cell line. This occurs because the plasmids utilize identical regulatory mechanisms for their replication and segregation. Consequently, one plasmid will eventually be lost from the population as cells divide, ensuring that only one type of related plasmid remains in cell.

**233.** (A) F plasmids (Fertility)
Fertility plasmids, or F plasmids, are a specialized class of bacterial DNA elements that enable conjugation. They encode the genetic information necessary for the synthesis of the conjugative pilus and the machinery required to transfer a copy of the plasmid to a recipient cell. The presence of an F plasmid defines the donor cell as F$^+$, facilitating horizontal gene transfer.

**234.** (A) Lack of histones and proximity to reactive oxygen species
The mitochondrial genome exhibits a significantly higher mutation rate than nuclear DNA. This increased susceptibility to damage is due to several factors, including the lack of protective histone proteins and the close physical proximity of the mtDNA to the electron transport chain, which generates reactive oxygen species. Furthermore, mitochondria have less efficient DNA repair mechanisms than the nuclear compartment.

**235.** (A) Heteroplasmy
Heteroplasmy is a genetic condition where a single cell or individual contains a mixture of different mitochondrial DNA genotypes. This often occurs when a mutation arises in one of the many mtDNA molecules within a mitochondrion. The ratio of mutant to wild-type mtDNA can vary between tissues and over time, leading to significant differences in the severity of mitochondrial diseases.

**236.** (A) Regulatory elements at the origin of replication (oriV)
The copy number of a bacterial plasmid is strictly regulated by specific genetic elements located at its origin of replication, oriV. These regulatory systems, which often involve small RNAs or proteins, ensure that the plasmid replicates at a rate consistent with the growth of the host cell. This prevents the plasmid from either being lost or overwhelming the cellular machinery.

**237.** (D) Leber Hereditary Optic Neuropathy (LHON)
Leber Hereditary Optic Neuropathy is a quintessential example of a human disorder caused by mutations in the mitochondrial genome. It is characterized by the sudden loss of central vision due to the degeneration of retinal ganglion cells. As a mitochondrially inherited disease, it is passed exclusively from the mother to all her children, demonstrating the unique maternal inheritance pattern.

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**238.** (C) Degradative plasmids
Degradative plasmids are specialized extrachromosomal elements that provide bacteria with the ability to metabolize complex and often toxic environmental compounds, such as toluene, salicylic acid, or pesticides. These plasmids encode the enzymes required for the breakdown of these substances into simpler molecules that can be used for energy. They are essential for the survival of bacteria in contaminated.

**239.** (A) 13 critical components of oxidative phosphorylation
The human mitochondrial genome is highly efficient and compact, encoding a total of 13 proteins. All of these proteins are essential subunits of the enzyme complexes involved in oxidative phosphorylation and the electron transport chain. In addition to these proteins, the mtDNA encodes 22 transfer RNAs and 2 ribosomal RNAs required for the independent translation of these mitochondrial subunits.

**240.** (A) The Endosymbiotic Theory
The endosymbiotic theory provides a widely accepted explanation for the evolutionary origin of mitochondria and chloroplasts in eukaryotic cells. It suggests that these organelles were originally free-living prokaryotes that were engulfed by a larger ancestral cell. Over time, the endosymbionts lost most of their genes to the host nucleus and became specialized organelles, retaining their own independent genomes.

**241.** (A) Insertion sequences (IS elements)
Insertion sequences are the simplest type of mobile genetic elements found in bacteria. They are small DNA segments that typically encode only the enzyme transposase, which is required for their mobilization. IS elements are characterized by short inverted repeats at their ends, which serve as recognition sites for the transposase. Their insertion into new genomic locations can disrupt gene.

**242.** (B) Short direct repeats flanking the inserted element
The integration of a transposable element into a new genomic location creates a characteristic structural hallmark known as short direct repeats. These repeats flank the inserted element and are formed because the transposase creates staggered nicks in the target DNA. After the transposon is inserted, the resulting single-stranded gaps are filled by DNA polymerase, duplicating the sequence at site.

**243.** (A) Central auxiliary genes flanked by two insertion sequences
Bacterial composite transposons, such as Tn10, are complex mobile elements formed by two insertion sequences flanking a central region of DNA. This central region often contains auxiliary genes, such as those providing antibiotic resistance. The entire unit moves as a single piece, utilizing the transposase encoded by one of the IS elements to catalyze the excision and integration steps.

**244.** (A) Ds elements are non-autonomous and rely on Ac for mobilization
In Barbara McClintock's Ac/Ds system in maize, the Ac element is autonomous because it encodes its own functional transposase. In contrast, the Ds element is non-autonomous and cannot mobilize on its own. Ds elements are typically derived from Ac elements through deletions that remove the transposase gene. They can only transpose when a functional Ac element is present.

**245.** (D) RNA intermediate followed by reverse transcription
Eukaryotic retrotransposons utilize a unique mobilization strategy that involves an RNA intermediate. The element is first transcribed into RNA, which is then reverse transcribed into a cDNA copy by a specialized enzyme. This new DNA copy is subsequently integrated into a different location in the genome. This copy-and-paste mechanism leads to an increase in the number of transposable elements.

**246.** (B) Short interspersed nuclear elements (SINEs)
Alu sequences are the most abundant type of mobile genetic elements in the human genome, belonging to the class of short interspersed nuclear elements. They are approximately 300 base pairs long and do not encode any proteins. Alu elements are non-autonomous retrotransposons that rely on the machinery of other elements, like LINEs, for their mobilization through an RNA.

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**247.** (B) Encode a functional reverse transcriptase and endonuclease
Long interspersed nuclear elements are considered autonomous retrotransposons because they

encode the necessary enzymes for their own mobilization. Specifically, a functional LINE-1 element encodes a protein with both reverse transcriptase and endonuclease activities. These enzymes allow the element to transcribe its own RNA, generate a DNA copy, and integrate it into a new genomic location without other assistance.

**248.** (A) Cointegrate
During the process of replicative transposition, such as that utilized by the Tn3 transposon, the donor and target DNA molecules are temporarily fused together. This large, intermediate structural complex is known as a cointegrate. It contains two copies of the transposable element, one at each junction between the two original DNA molecules, and must be resolved to complete transposition.

**249.** (B) Resolvase
The resolution of a cointegrate intermediate in replicative transposition is catalyzed by a specialized enzyme called resolvase. This site-specific recombinase recognizes internal sequences within the two copies of the transposon and promotes a crossover event. This reaction separates the two DNA molecules, leaving one copy of the transposon at the original site and another copy at the target site.

**250.** (B) Retroviruses
Long Terminal Repeat retrotransposons are a class of mobile elements that are structurally and evolutionarily related to retroviruses. Like viruses, they contain long terminal repeats and encode enzymes such as reverse transcriptase and integrase. The primary difference is that LTR retrotransposons lack the envelope genes necessary to form infectious particles and leave the host cell, instead replicating solely within.

**251.** (A) Null mutation causing loss of gene function
When a transposable element integrates directly into the coding sequence of an essential gene, it typically causes a null mutation. The large insertion disrupts the reading frame or the physical structure of the gene, leading to the production of a non-functional protein or a complete loss of gene expression. This results in the total inactivation of the gene's biological.

**252.** (D) Transposon insertion into a pigment gene and subsequent excision
Genetic variegation, such as the spotted patterns in maize kernels, is a phenotypic result of transposable element activity. It occurs when a transposon that has inactivated a pigment gene excises itself during the development of a tissue. The excision restores the gene's function in a subset of cells, leading to clones of pigmented cells that appear as spots.

**253.** (A) Variable Number Tandem Repeats (VNTRs)
Minisatellites, also known as Variable Number Tandem Repeats, are genomic regions characterized by the tandem repetition of a specific DNA sequence, typically 10 to 100 base pairs in length. These loci are highly polymorphic in the human population, as the number of repeats varies significantly between individuals. This variability makes VNTRs a powerful tool for mapping and forensic analysis.

**254.** (A) SSRs have 1-6 bp repeats, while VNTRs have 10-100 bp
Microsatellites and minisatellites are both types of tandemly repeated DNA but differ in the size of their repeat units. Microsatellites, or short tandem repeats, consist of very short sequences, typically one to six base pairs long. Minisatellites have longer repeat units, ranging from ten to one hundred base pairs. Both are highly polymorphic and widely used as genetic.

**255.** (C) Slipped-strand mispairing (replication slippage) during DNA replication
The high rate of length variation in microsatellite tracts is primarily caused by a molecular mechanism known as slipped-strand mispairing, or replication slippage. During DNA synthesis, the nascent and template strands can momentarily dissociate and then reanneal out of register due to the repetitive nature of the sequence. This creates a small loop that results in additions or deletions.

**256.** (A) An expansion (increase) in the number of tandem repeats
If replication slippage leads to the formation of a loop in the newly synthesized daughter strand, the net result after the next round of replication is an expansion of the microsatellite tract. The DNA polymerase effectively adds extra repeat units to the sequence. This process is a common

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source of genetic diversity and is responsible for certain hereditary trinucleotide.

**257.** (B) Huntington's Disease
Huntington's Disease is a devastating neurological disorder caused by the abnormal expansion of a CAG trinucleotide repeat within the HTT gene. This expansion results in an abnormally long polyglutamine tract in the huntingtin protein, which becomes toxic and leads to the progressive degeneration of neurons in the brain. The disease exhibits genetic anticipation, where severity increases in successive generations.

**258.** (B) Telomeres
Telomeres are specialized tandem repeat arrays located at the extreme ends of linear eukaryotic chromosomes. In humans, they consist of the repeating sequence TTAGGG. Telomeres serve as protective caps that prevent the chromosome ends from being recognized as DNA damage and compensate for the end-replication problem. Their gradual shortening over time is linked to cellular aging and biological senescence.

**259.** (A) Highly variable restriction fragment lengths between individuals
Variable Number Tandem Repeats are exceptionally useful in classical DNA fingerprinting because of their high degree of polymorphism. The number of repeats at a specific locus varies among individuals, leading to differences in the length of DNA fragments produced by restriction enzyme digestion. By comparing the patterns of these fragments, scientists can create a unique genetic profile for forensic identification.

**260.** (B) Alpha-satellite (alphoid) DNA
Alpha-satellite DNA, also known as alphoid DNA, is the primary structural component of functional centromeres in higher primates, including humans. It consists of a basic 171-base pair repeat unit organized into large tandem arrays. These sequences are essential for the recruitment of centromere-specific proteins and the assembly of the kinetochore, ensuring proper chromosome segregation during the process of mitosis.

**261.** (A) Exert no significant phenotypic effect
Mutations that alter the length of microsatellite tracts in non-regulatory intronic regions generally persist in a population because they exert no significant phenotypic effect. Since these changes do not affect the protein sequence or the regulation of gene expression, they are not subject to negative natural selection. Consequently, these polymorphic markers accumulate over time and are useful for studying diversity.

**262.** (A) Short Tandem Repeats (STRs)
Forensic scientists heavily utilize Short Tandem Repeats for DNA profiling because they are highly polymorphic and can be easily amplified by PCR. The small size of STR repeat units allows for the analysis of even degraded DNA samples. By multiplexing the amplification of several different STR loci, investigators can generate a genetic fingerprint with an extremely high probability of.

**263.** (A) Misalignment of chromatids along tandemly repeated DNA
Unequal crossing over is a type of homologous recombination error that occurs when chromatids misalign during meiosis. This misalignment is typically facilitated by the presence of tandemly repeated DNA sequences. When a crossover event occurs between these misaligned regions, the resulting chromosomes have an unequal number of repeats, leading to the duplication of genes on one chromosome and deletions on.

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**264.** (A) In the 5'-UTR, leading to hypermethylation and silencing
Fragile X syndrome is a genetic disorder caused by the expansion of a CGG trinucleotide repeat in the 5' untranslated region of the FMR1 gene. When the number of repeats exceeds a critical threshold, the region becomes hypermethylated, leading to the transcriptional silencing of the gene. The resulting lack of the FMRP protein is responsible for the developmental symptoms.

**265.** (A) Mobile junction involving reciprocal strand exchange between homologs
The Holliday junction is a fundamental structural intermediate in homologous recombination, characterized by a cross-shaped structure. It forms when two homologous DNA duplexes undergo reciprocal strand exchange, creating a mobile junction where the four strands are held together. This structure allows the exchange of genetic information between chromosomes and must be resolved to complete the process of homologous.

**266.** (D) Branch migration
Following the initial strand invasion and formation of the Holliday junction, the process of branch migration occurs. In this step, the crossover point moves along the DNA, extending the region of heteroduplex DNA where strands from the two different chromosomes are paired. This migration is essential for the exchange of significant amounts of genetic information between the homologous DNA molecules.

**267.** (A) RecBCD enzyme complex
The RecBCD enzyme complex is the primary initiator of homologous recombination at double-strand breaks in E. coli. It possesses both helicase and nuclease activities, allowing it to unwind and degrade the DNA ends. RecBCD processes the DNA until it encounters a specific Chi site, which triggers a change in its activity that is essential for subsequent recombination steps in.

**268.** (C) Preferential 5' strand degradation, creating a 3' overhang
When the RecBCD complex encounters a Chi site, its nuclease activity is altered. The enzyme stops degrading the 3'-ending strand and increases the degradation of the 5'-ending strand. This results in the generation of a single-stranded DNA segment with a 3' overhang. This specific tail is a crucial substrate for the RecA protein, which facilitates the subsequent strand invasion steps.

**269.** (B) RecA
The single-stranded 3' overhang generated by the RecBCD complex is rapidly coated by the RecA protein. RecA forms a helical nucleoprotein filament that searches for a homologous DNA sequence. Once a match is found, RecA catalyzes the invasion of the single strand into the homologous duplex, initiating the formation of a D-loop and the subsequent steps of recombination.

**270.** (A) RuvAB
Branch migration during homologous recombination in E. coli is actively driven by the RuvAB complex. RuvA recognizes and binds to the Holliday junction, while RuvB functions as an ATP-dependent motor protein that pulls the DNA strands through the junction. This coordinated action allows the crossover point to move rapidly along the DNA, facilitating the exchange of genetic information.

**271.** (B) RuvC
The final step of homologous recombination in bacteria involves the resolution of the Holliday junction, which is performed by the RuvC endonuclease. RuvC specifically recognizes the junction and cleaves two of the four DNA strands at a conserved sequence. This cleavage allows the two DNA molecules to separate, completing the physical exchange of genetic material between homologous chromosomes.

**272.** (A) Crossover (splice) or non-crossover (patch) products
The resolution of a Holliday junction by RuvC can lead to two different genetic outcomes depending on which strands are cleaved. If the original invading strands are cut, the result is a non-crossover, or patch, product. If the non-invading strands are cut, a crossover, or splice, product is formed. Both outcomes involve the exchange of genetic information within heteroduplexes.

**273.** (A) Two adjacent Holliday junctions
The Double-Strand Break Repair model is an updated framework for understanding homologous recombination. A signature feature of this model is the formation of two adjacent Holliday junctions following the invasion of both ends of a double-strand break into a homologous duplex. The resolution of these two junctions can result in either crossover or non-crossover products, depending on cleavage planes.

**274.** (A) Spo11
During the prophase of meiosis I, the initiation of homologous recombination is triggered by the deliberate creation of double-strand breaks. In eukaryotic cells, this process is catalyzed by a specialized endonuclease called Spo11. By generating these breaks at specific locations, Spo11 ensures that recombination and crossing over occur between homologous chromosomes, which is essential for proper segregation and genetic diversity.

**275.** (A) Mismatch Repair (MMR)
Gene conversion is a non-reciprocal genetic exchange where one DNA sequence is replaced by a homologous sequence. This process most commonly occurs during the repair of mismatches within the heteroduplex DNA formed during recombination. When the mismatch repair machinery corrects these base-pair differences using one strand as a template, the genetic

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information from one chromosome is effectively converted to other.

**276.** (C) Initial single-strand invasion into a homologous duplex
The displacement loop, or D-loop, is a critical structural intermediate that forms during the initial stages of homologous recombination. It is created when a single-stranded DNA end, coated with recombinase proteins like RecA or Rad51, invades a homologous double-stranded DNA molecule. The invading strand base-pairs with its complement, displacing the other strand of the duplex and creating stable.

**277.** (A) Takes up free extracellular DNA from the environment
Bacterial transformation is a process of horizontal gene transfer where a recipient cell directly takes up free, naked DNA from the surrounding extracellular environment. This DNA, often released by the lysis of other bacteria, can then be integrated into the recipient's genome through homologous recombination. Transformation allows bacteria to acquire new genetic traits, such as antibiotic resistance, from surroundings.

**278.** (B) Competent
Natural transformation only occurs when bacterial cells are in a specialized physiological state known as competence. This state is often induced by specific environmental conditions or signaling molecules. Competent cells express the proteins required for binding extracellular DNA, transporting it across the cell membrane, and protecting it from degradation during its integration into the bacterial chromosome or.

**279.** (B) One strand is degraded, and a single strand enters the cytoplasm
During the process of natural transformation in many bacteria, double-stranded DNA from the environment binds to the cell surface. As the DNA is transported into the cytoplasm, one of the strands is typically degraded by membrane-bound nucleases, and only a single strand enters the cell. This single-stranded fragment then serves as a substrate for recombination with the host's chromosome.

**280.** (D) Physical cell-to-cell contact via a conjugative pilus
Bacterial conjugation is a mechanism of horizontal gene transfer that fundamentally requires direct physical contact between a donor and a recipient cell. This contact is mediated by a specialized structure called a conjugative pilus, which is synthesized by the donor. The pilus brings the two cells into close proximity, allowing for the formation of a bridge through DNA.

**281.** (A) Receives a single-stranded copy of F plasmid and becomes $F^+$
During a standard $F^+$ x $F^-$ conjugation event, the donor cell ($F^+$) transfers a single-stranded copy of the fertility plasmid to the recipient cell ($F^-$). Once the transfer is complete, both cells synthesize the complementary strand to reform the circular double-stranded plasmid. As a result, the recipient cell is converted into a functional $F^+$ donor capable of further transfer.

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**282.** (A) oriT (origin of transfer)
The transfer of the $F^-$factor during bacterial conjugation begins at a specific DNA sequence known as the origin of transfer, or oriT. A specialized enzyme called a relaxase creates a site-specific nick in one strand of the DNA at this location. The resulting single strand is then unwound and directed into the recipient cell through the conjugation bridge.

**283.** (C) $F^-$factor integrated into the host chromosome
An Hfr, or High Frequency of Recombination, strain is a specialized type of E. coli where the fertility factor has integrated into the bacterial chromosome. Because the $F^-$factor is now part of the much larger chromosome, conjugation in Hfr strains involves the transfer of chromosomal genes along with the plasmid sequence. This integration allows for the efficient mapping of chromosomal.

**284.** (A) Relative linear order and distance of genes on the chromosome
Interrupted mating experiments are a classic technique used to map the relative positions of genes on the bacterial chromosome. By periodically stopping conjugation between an Hfr donor and an $F^-$ recipient, researchers can determine the sequence in which different donor alleles enter the recipient cell. The time of entry for each gene reflects its relative linear distance from origin.

**285.** (D) Conjugation bridge breaks before the entire chromosome is transferred
In most Hfr x $F^-$ conjugation events, the recipient cell remains functionally $F^-$ even after receiving donor DNA. This occurs because the entire bacterial chromosome is very large, and the conjugation bridge typically breaks before the complete $F^-$factor sequence is transferred. As a result, the recipient does not acquire all the genes necessary to become a donor, although genes.

**286.** (B) Rolling-circle replication
The transfer of DNA during bacterial conjugation utilizes a specialized mechanism known as rolling-circle replication. As one strand of the $F^-$plasmid is nicked and moved into the recipient cell, DNA polymerase in the donor cell simultaneously synthesizes a new complementary strand to replace it. This ensures that the donor cell retains a complete copy of the genetic material throughout.

**287.** (B) An F' ($F^-$prime) plasmid
An F' ($F^-$prime) plasmid is formed when an integrated $F^-$factor in an Hfr cell undergoes imprecise excision from the bacterial chromosome. During this process, the plasmid loop carries along some adjacent chromosomal genes. When an F' plasmid is transferred during conjugation, the recipient cell receives these specific chromosomal genes along with the fertility factor, creating a partial.

**288.** (A) Merodiploid (partial diploid)
A merodiploid is a partially diploid bacterial cell that contains two copies of certain genes—one on the primary chromosome and another on an extrachromosomal element like an F' plasmid. This state is typically created through the conjugation of an F' donor with an $F^-$ recipient. Merodiploids are valuable in genetic research for studying gene regulation, dominance, and allele.

**289.** (A) Random host chromosome fragments packaged into a viral capsid
Generalized transduction is a mechanism of horizontal gene transfer mediated by bacteriophages. It occurs when random fragments of the bacterial donor's chromosome are accidentally packaged into a viral capsid instead of the phage genome during the lytic cycle. When this defective phage infects a new recipient cell, it injects the bacterial DNA, which can then be integrated into host.

**290.** (B) Host chromosome is shattered into phage-sized fragments and packaged
In generalized transduction, any gene from the donor bacterial chromosome has an equal chance of being transferred. This is because the phage-encoded enzymes shatter the host chromosome into many small pieces during the infection process. Some of these fragments, which are roughly the same size as the phage genome, are mistakenly packaged into the newly formed viral heads.

**291.** (A) Host genes located immediately adjacent to the prophage integration site
Specialized transduction is a highly specific mechanism of gene transfer that involves temperate phages like lambda. It can only transfer bacterial genes that are located immediately adjacent to the prophage integration site on the chromosome. This occurs during the imprecise excision of the prophage, where a piece of host DNA is removed along with a portion of viral.

**292.** (D) gal and bio
In the E. coli chromosome, the specialized site for the integration of the lambda prophage, known as attB, is located at a precise position between the galactose (gal) and biotin (bio) operons. Because of this specific localization, specialized transduction by lambda phages is restricted to the transfer of genes from these two neighboring metabolic pathways between different cells.

**293.** (A) Lambda Integrase (Int)
The integration of the lambda phage genome into the E. coli chromosome is a site-specific recombination event catalyzed by the viral enzyme lambda integrase. This protein recognizes the attachment sites on both the viral and bacterial DNA, facilitating their crossover and fusion. This process allows the phage to enter the lysogenic cycle, where it remains dormant as a.

**294.** (D) Lysogen
A lysogen is a bacterial cell that carries a dormant viral genome, known as a prophage, integrated into its own chromosome. In this state, the phage genes are mostly repressed, and the viral DNA is replicated along with the host DNA during

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each cell division. A lysogen is immune to further infection by the same type of phage.

**295.** (A) SOS response to DNA damage, leading to viral repressor cleavage
The transition of a lambda prophage from the dormant lysogenic state to the active lytic cycle is most commonly induced by the bacterial SOS response. When the host cell suffers DNA damage, the RecA protein is activated and promotes the selF cleavage of the lambda repressor. Without the repressor, the viral genes for replication and lysis are expressed rapidly.

**296.** (A) cI (lambda repressor) and Cro
The decision between the lytic and lysogenic pathways in lambda phage infection is determined by the balance between two regulatory proteins: the cI repressor and the Cro protein. The cI repressor promotes lysogeny by silencing the lytic genes, while Cro promotes the lytic cycle by inhibiting cI expression. The outcome of this molecular switch depends on environmental conditions.

**297.** (A) Defective, requiring a helper phage for replication and lysis
In specialized transduction, the imprecise excision of a prophage often results in a defective viral genome that lacks essential genes for replication or capsid formation. These defective phages can only complete their life cycle and infect new cells if a helper phage is present in the same cell to provide missing functions. This allows the transfer of adjacent bacterial.

**298.** (C) Excisionase (Xis)
The excision of the lambda prophage from the bacterial chromosome requires the coordinated action of two viral proteins: integrase and excisionase. While integrase is needed for both integration and excision, excisionase is only produced when the phage enters the lytic cycle. Xis directs the integrase to catalyze the recombination event at the junction sites, allowing viral release.

**299.** (A) Heteroduplex joints
During mixed infections with different strains of $T_4$ bacteriophages, recombination between the viral genomes frequently occurs. This process leads to the formation of unique structural intermediates known as heteroduplex joints. These are regions where the two strands of the DNA double helix originate from different parental phages. The repair and resolution of these joints contribute to viral genetic diversity.

**300.** (C) Genes located close together, within a single phage DNA headful
In bacterial genetics, the frequency at which two genes are cotransduced by a single bacteriophage is a direct measure of their physical proximity on the chromosome. Because a phage capsid has a limited capacity for DNA, only genes that are located very close together can be packaged into the same viral head. Therefore, high cotransduction frequency indicates tight linkage.

**********

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# CHAPTER 03

# Gene Structure and Regulation of Gene Expression

2016

**1.** The point on a Cot curve where half the DNA has reassociated is directly proportional to which parameter?
(A) Kinetic complexity of the genome
(B) GC content of the genome
(C) Optical density at 260 nm
(D) Rate of thermal denaturation

2009

**2.** Which factor primarily dictates the rate of DNA renaturation in a highly complex eukaryotic genome?
(A) Phosphate backbone charge
(B) Telomerase activity
(C) Concentration of unique sequences
(D) Ribosomal RNA abundance

2008

**3.** What is the most appropriate indicator of genome complexity derived from DNA reassociation kinetics?
(A) HalF-Cot value
(B) Melting temperature
(C) Hyperchromic shift
(D) Renaturation temperature

2018

**4.** Identify the INCORRECT statement regarding the Cot curve analysis of eukaryotic DNA.
(A) It reveals multiple components of differing repetition frequencies.
(B) Repetitive DNA reassociates at lower Cot values than unique DNA.
(C) Genome size influences the Cot value of the unique fraction.
(D) The fast-reassociating fraction represents unique sequences.

2001

**5.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Eukaryotic genomes display multiphasic Cot curves.
**Reason (R):** Eukaryotic genomes contain unique, moderately repetitive, and highly repetitive DNA sequences.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2025

**6.** The reassociation of which DNA fraction characterizes the lowest Cot value in a standard kinetics curve?
(A) Single-copy genes
(B) Transposable elements
(C) Satellite DNA
(D) Intronic sequences

2016

**7.** According to DNA reassociation kinetics, how is the sequence length of the repeating unit related to the renaturation rate?
(A) Positively correlated
(B) Exponentially correlated
(C) Completely independent
(D) Inversely proportional

2010

**8.** Which analytical finding directly emerges from examining the intermediate reassociating fraction of a eukaryotic Cot curve?
(A) Presence of LINEs and SINEs
(B) Total number of centromeres
(C) Rate of spontaneous mutation
(D) Length of the telomere overhang

2011

**9.** In assessing genome complexity, which type of sequences complicate the determination of true unique gene number?
(A) Exons of housekeeping genes
(B) Pseudogenes and paralogs
(C) Promoters of structural genes
(D) Origin of replication sites

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2005

**10.** The concept of genome equivalence suggests that all somatic cells contain the same DNA. Which phenomenon complicates interpreting genome complexity based solely on Cot curves?
(A) Somatic hypermutation and allelic exclusion
(B) Polyploidy and genome duplication
(C) Alternative splicing and isoform variety
(D) Post-translational modification of histones

2007

**11.** Which class of DNA sequence is predominantly located in centromeric heterochromatin?
(A) Highly repetitive satellite DNA
(B) Unique structural genes
(C) Short interspersed nuclear elements
(D) Long interspersed nuclear elements

2010

**12.** Alu elements in the human genome are a classic example of which classification?
(A) Highly repetitive DNA
(B) LINEs
(C) SINEs
(D) Unique DNA

2024

**13.** Which feature distinguishes LINEs from SINEs in eukaryotic genomes?
(A) SINEs are autonomous elements encoding all necessary enzymes.
(B) SINEs encode their own reverse transcriptase for mobility.
(C) LINEs lack any protein-coding sequences for transposition.
(D) LINEs encode proteins for their own retrotransposition.

2024

**14.** What is the fundamental evolutionary mechanism driving the expansion of moderately repetitive DNA sequences like LINEs?
(A) DNA-mediated conservative recombination
(B) Homologous end joining
(C) RNA-mediated transposition
(D) Telomere elongation

2016

**15.** Match the DNA sequence classifications in List I with their primary features in List II.
List I: (DNA Sequence Classifications)
P. SINEs
Q. Satellite DNA
R. Unique DNA
S. LINEs
List II: (Primary Features)
1. Tandemly repeated in centromeres
2. Contains structural genes
3. Non-autonomous retrotransposons
4. Autonomous retrotransposons
(A) P-1, Q-2, R-3, S-4
(B) P-3, Q-1, R-2, S-4
(C) P-4, Q-3, R-1, S-2
(D) P-2, Q-4, R-3, S-1

2011

**16.** Identify the correct pair regarding repetitive DNA and its general genomic location.
(A) Satellite DNA – Confined strictly to telomeric and sub-telomeric domains
(B) Minisatellites – Found only within localized centromeric heterochromatin
(C) SINEs – Located exclusively within the coding regions of structural exons
(D) Microsatellites – Dispersed throughout the genome

2013

**17.** Which statement INCORRECTLY characterizes highly repetitive DNA?
(A) It is highly transcribed into mRNA.
(B) It constitutes a major fraction of the eukaryotic genome.
(C) It usually consists of short tandem repeats.
(D) It separates as a distinct band in density gradient centrifugation.

2007

**18.** The classical genetic concept of the "cistron" is functionally equivalent to which modern molecular entity?
(A) A single nucleotide polymorphism
(B) A transcription factor
(C) A structural gene encoding a polypeptide
(D) A continuous reading frame without introns

2003

**19.** Seymour Benzer introduced the term "muton." What does this term specifically define?
(A) The smallest sequence of DNA capable of mutation
(B) The unit of genetic recombination
(C) The minimum unit of genetic function
(D) The smallest unit of DNA capable of independent replication

2010

**20.** Which term refers to the smallest functional unit of recombination within a gene?
(A) Muton
(B) Cistron
(C) Replicon

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(D) Recon

2021
**21.** How did the discovery of split genes modify the classical definition of a cistron?
(A) It proved that regulatory sequences are always absent from coding segments.
(B) It showed that a single continuous sequence is not required to encode a protein.
(C) It demonstrated that all structural genes must be transcribed simultaneously.
(D) It indicated that prokaryotic and eukaryotic organisms share identical gene structures.

2016
**22.** Which evolutionary advantage is most directly provided by the split gene architecture in eukaryotes?
(A) Prevention of harmful spontaneous mutations within the genome
(B) Faster replication of the large eukaryotic genomic structure
(C) Increased protein diversity through alternative splicing
(D) Elimination of the complex requirement for post-transcriptional RNA processing

2025
**23.** What is the fundamental cause of the "c-value paradox" in eukaryotes?
(A) Variations in non-coding and repetitive DNA content
(B) Variations in the total number of essential metabolic genes
(C) Discrepancies in DNA polymerase processivity during elongation
(D) Unequal rates of protein synthesis between different organisms

2021
**24.** Which structural component is absolutely essential to define the boundaries of a basic transcription unit?
(A) Enhancer and silencer
(B) Promoter and terminator
(C) Operator and attenuator
(D) Intron and exon

2014
**25.** Given below are three statements regarding a typical eukaryotic transcription unit:
I. The coding sequence is entirely continuous.
II. A promoter is located upstream of the transcription start site.
III. Terminators dictate the end of RNA synthesis.
Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I, II and III

2010
**26.** Which functional consequence arises from the presence of a strong promoter in a transcription unit?
(A) Frequent initiation of transcription
(B) Immediate degradation of the transcript
(C) Complete suppression of translation
(D) Rapid termination of elongation

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2023
**27.** Identify the INCORRECT pair regarding transcription unit components.
(A) Promoter – Binding site for RNA polymerase
(B) Terminator – Signals dissociation of the transcription complex
(C) Transcription start site – The +1 nucleotide
(D) Coding sequence – Exclusively contains regulatory elements

2003
**28.** In which genomic context do overlapping genes occur most frequently?
(A) Large eukaryotic genomes of multicellular organisms
(B) Mammalian mitochondrial DNA within specialized organelles
(C) Viral genomes like bacteriophage phiX174
(D) Plant chloroplast genomes found in photosynthetic tissue

2002
**29.** What is the primary evolutionary driver for the existence of overlapping genes in bacteriophages?
(A) Enhancing metabolic complexity through gene fusion events
(B) Maximizing information storage in restricted genome sizes
(C) Increasing the rate of genetic recombination during infection
(D) Preventing viral genome integration into host chromosomes

2009
**30.** How do overlapping genes typically encode distinct functional proteins from the same DNA segment?
(A) By utilizing alternate translation start sites and frames
(B) By using unique sets of specialized tRNA molecules for each gene

(C) By reversing the thermodynamic direction of the DNA double helix
(D) By bypassing the transcription step via direct translation of DNA

2003
**31.** Which genetic phenomenon is most constrained by the presence of overlapping genes?
(A) Frequency of silent mutations
(B) Speed of transcriptional initiation
(C) Frequency of transposon mobility
(D) Evolutionary sequence divergence

2021
**32.** In bacteriophage phiX174, the phenomenon of overlapping genes demonstrates which fundamental concept?
(A) One sequence can harbor multiple open reading frames.
(B) The genetic code is non-degenerate in viral systems.
(C) The universality of the genetic code is largely false.
(D) Viral replication requires eukaryotic host polymerases.

2010
**33.** Which component of the prokaryotic RNA polymerase holoenzyme confers specific promoter recognition?
(A) Alpha subunit
(B) Sigma factor
(C) Beta subunit
(D) Omega subunit

2023
**34.** What is the primary functional role of the beta and beta-prime subunits in the prokaryotic RNA polymerase core enzyme?
(A) Recognizing the -10 and -35 consensus sequences
(B) Assembling the transcription termination hairpin
(C) Exporting the mRNA to the cytoplasm
(D) Catalyzing the formation of phosphodiester bonds

2006
**35.** Given below are four statements concerning prokaryotic sigma factors:
I. Sigma 70 is the primary housekeeping factor in E. coli.
II. Sigma factors remain attached to the core enzyme throughout elongation.
III. Different sigma factors activate distinct sets of genes under stress conditions.
IV. Sigma factors possess intrinsic helicase activity.
Which of the above statement(s) is/are correct?
(A) I and III
(B) I, II and IV
(C) II and IV
(D) I, III and IV

2011
**36.** Which process represents a critical functional distinction between the core enzyme and the holoenzyme of prokaryotic RNA polymerase?
(A) Synthesis of ribosomal RNA
(B) Processive transcript elongation
(C) Specific binding to promoter sequences
(D) Proofreading of the nascent RNA

2021
**37.** Which sigma factor is primarily responsible for transcribing heat shock genes in Escherichia coli?
(A) Sigma 32
(B) Sigma 28
(C) Sigma 54
(D) Sigma 70

2015
**38.** The transition from a closed promoter complex to an open promoter complex during prokaryotic transcription initiation involves:
(A) Full dissociation of the core enzyme from the promoter region
(B) Binding of the rho protein to the nascent RNA transcript
(C) Phosphorylation of the C-terminal domain of the polymerase
(D) Unwinding of DNA around the transcription start site

2016
**39.** What is the primary characteristic of abortive initiation during prokaryotic transcription?
(A) Release of short RNA transcripts before promoter escape
(B) Premature termination mediated by the rho termination factor
(C) Failure of the sigma factor to bind to the promoter sequence
(D) Inability of the cellular ribosome to translate the mRNA

2017
**40.** During the elongation cycle of prokaryotic transcription, what mechanism ensures high processivity?
(A) Continuous binding of the sigma factor
(B) The topological stability of the transcription

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(C) Frequent dissociation and rebinding of the polymerase
(D) Action of topoisomerase II directly on the RNA transcript

2018
**41.** Which structural feature is essential for intrinsic (rho-independent) termination of transcription?
(A) A GC-rich hairpin followed by a poly-U sequence in the RNA
(B) A poly-A tail added post-transcriptionally to the 3' end
(C) An AT-rich region followed by a GC-rich hairpin structure
(D) A specific binding site for the rho helicase on the RNA

2006
**42.** How does the rho protein facilitate transcription termination?
(A) It acts as a ribonuclease that degrades the emerging transcript.
(B) It binds the rut site as an ATP-dependent RNA-DNA helicase.
(C) It blocks the entry of nucleoside triphosphates to the site.
(D) It methylates the RNA transcript to induce complex dissociation.

2025
**43.** Which mechanistic factor fundamentally differentiates rho-dependent from rho-independent termination?
(A) The requirement for a trans-acting protein factor
(B) The involvement of the RNA polymerase core enzyme
(C) The synthesis of a phosphodiester bond in elongation
(D) The requirement for a stop codon in the reading frame

2004
**44.** What is the defining consequence of a mutation that deletes the rut site in a prokaryotic gene?
(A) Transcription initiation is permanently blocked.
(B) Intrinsic termination occurs prematurely.
(C) The sigma factor cannot dissociate.
(D) Rho-dependent termination fails to occur.

2004
**45.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Eukaryotic RNA polymerase II is highly sensitive to alpha-amanitin.
**Reason (R):** Alpha-amanitin specifically binds to RNA polymerase II and prevents RNA elongation.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2007
**46.** Which specific target transcript is synthesized by eukaryotic RNA polymerase I?
(A) All cellular messenger RNA (mRNA) sequences
(B) Specialized transfer RNA (tRNA) molecules
(C) 28S, 18S, and 5.8S ribosomal RNA (rRNA)
(D) Small regulatory micro RNA (miRNA) species

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2003
**47.** Which statement about eukaryotic RNA polymerase III is INCORRECT?
(A) It synthesizes 5S rRNA and tRNAs.
(B) It is insensitive or moderately sensitive to alpha-amanitin depending on the species.
(C) Its promoters are frequently located entirely downstream of the start site.
(D) It requires the TATA-binding protein exclusively for termination.

2015
**48.** What structural complexity is unique to eukaryotic RNA polymerases compared to the prokaryotic core enzyme?
(A) They completely lack a catalytic magnesium ion in the active site.
(B) They are composed of a single massive polypeptide chain structure.
(C) They contain 10-14 subunits with species-specific components.
(D) They possess an intrinsic sigma-like domain for promoter binding.

2016
**49.** Sensitivity to alpha-amanitin is widely used to classify eukaryotic RNA polymerases. Which polymerase is completely insensitive to this toxin at high concentrations?
(A) RNA Polymerase I
(B) RNA Polymerase II
(C) RNA Polymerase III
(D) Mitochondrial RNA Polymerase

2006
**50.** In the eukaryotic core promoter, the TATA box is typically located at which relative position to the transcription start site?
(A) -25 to -30
(B) +1
(C) -10
(D) -70 to -80

2014
**51.** Which basal transcription factor contains the TATA-binding protein (TBP) as a subunit?
(A) TFIIA
(B) TFIID
(C) TFIIB
(D) TFIIH

2002
**52.** The Downstream Promoter Element (DPE) is typically found in which type of eukaryotic promoters?
(A) Prokaryotic operon promoters
(B) RNA polymerase I promoters
(C) TATA-less promoters
(D) Enhancer regions

2016
**53.** What is the essential enzymatic function provided by TFIIH during the formation of the eukaryotic pre-initiation complex?
(A) RNA splicing
(B) DNA ligase activity
(C) RNA processing and polyadenylation
(D) Helicase and kinase activity

2019
**54.** The Initiator (Inr) element in eukaryotic promoters encompasses which functional region?
(A) The translation start codon
(B) The first intron-exon boundary
(C) The termination signal
(D) The transcription start site

2003
**55.** Match the eukaryotic regulatory element or factor in List I with its specific function in List II.
List I: (Regulatory Element/Factor)
P. Enhancer
Q. Insulator
R. Silencer
S. TBP
List II: (Specific Function)
1. Binds the TATA box
2. Increases transcription rate independent of orientation
3. Blocks the interaction between enhancers and inappropriate promoters
4. Actively represses transcription
(A) P-2, Q-3, R-4, S-1
(B) P-3, Q-2, R-1, S-4
(C) P-1, Q-4, R-2, S-3
(D) P-4, Q-1, R-3, S-2

2012
**56.** Which defining characteristic best describes eukaryotic enhancer sequences?
(A) They are exclusively transcribed into functional non-coding RNAs in eukaryotes.
(B) They function in an orientation and distance-independent manner.
(C) They must be located within 50 base pairs of the promoter sequence to work.
(D) They act only on RNA polymerase III promoters to increase the reaction rate.

2014
**57.** By what molecular mechanism do insulator elements typically function?
(A) By establishing physical boundaries in chromatin looping
(B) By recruiting DNA methyltransferases to promoters
(C) By directly degrading mRNA transcripts
(D) By preventing the binding of general transcription factors

2004
**58.** How does a eukaryotic silencer element exert its repressive effect on transcription?
(A) By recruiting corepressors that induce repressive chromatin marks.
(B) By acting as a competitive inhibitor of the RNA polymerase II enzyme.
(C) By directly cutting the nascent RNA transcript as it leaves the site.
(D) By unwinding the DNA double helix at the TATA box in the promoter.

2025
**59.** Which of the following DNA-binding domains typically utilizes an alpha helix inserted into the major groove of the DNA?
(A) Zinc finger
(B) Leucine zipper
(C) Helix-turn-helix
(D) Homeodomain

2014
**60.** The zinc finger motif is stabilized by the coordination of a zinc ion to which specific amino acid residues?

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(A) Cysteine and Histidine
(B) Arginine and Lysine
(C) Serine and Threonine
(D) Glutamate and Aspartate

2011

**61.** What is the primary structural basis for DNA binding in a leucine zipper motif?
(A) A beta-sheet structure that inserts into the minor groove for stability.
(B) Dimerization of alpha-helices facilitating DNA interaction.
(C) Coordination of a metal ion to stabilize an unstructured loop structure.
(D) Covalent linkage to the DNA backbone via a specialized enzymatic process.

2010

**62.** Which structural characteristic is essential for the function of a basic helix-loop-helix (bHLH) transcription factor?
(A) The presence of a catalytic ribozyme domain within the protein sequence.
(B) The mandatory binding of a zinc ion to stabilize the secondary structure.
(C) A specific requirement for RNA as a cofactor for DNA binding activity.
(D) A basic region for DNA binding and an HLH domain for dimerization.

2022

**63.** Which modification constitutes the 5' capping of eukaryotic mRNA?
(A) Addition of 7-methylguanosine via a 5'-5' triphosphate link.
(B) Attachment of a long poly-A tail to the 3' end of the transcript.
(C) Methylation of the 2'-OH group of the first intron in the sequence.
(D) Cleavage of the transcript by a specialized nuclear endonuclease.

2014

**64.** What is the primary biological function of the 5' mRNA cap?
(A) It prevents 5' to 3' degradation and aids translation.
(B) It dictates the site of alternative splicing in eukaryotes.
(C) It signals for the termination of the transcription process.
(D) It provides the energy required for the elongation cycle.

2024

**65.** Given below are three statements regarding 3' polyadenylation of eukaryotic mRNA:
I. It requires the consensus sequence AAUAAA.
II. Poly-A polymerase requires an RNA template.
III. It protects the 3' end from rapid exonucleolytic degradation.
Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I, II and III

2001

**66.** The 3' cleavage of the pre-mRNA transcript occurs generally at what position relative to the AAUAAA signal?
(A) 10-30 nucleotides upstream
(B) 10-30 nucleotides downstream
(C) Exactly at the signal
(D) 100-200 nucleotides downstream

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2006

**67.** Which process fundamentally distinguishes mRNA maturation in eukaryotes from that in prokaryotes?
(A) Simultaneous transcription and translation of genetic material.
(B) Extensive capping, splicing, and polyadenylation of transcripts.
(C) Utilization of the same RNA polymerase for all cellular RNAs.
(D) Absence of any non-coding regions within the mature mRNA structure.

2006

**68.** The spliceosome is primarily composed of which molecular entities?
(A) Transfer RNAs (tRNAs) and a specific set of ribosomal proteins.
(B) MicroRNAs (miRNAs) and the associated RISC protein complex.
(C) Small nuclear RNAs (snRNAs) and associated proteins (snRNPs).
(D) DNA polymerases and ligases required for genetic repair events.

2016

**69.** Which specific sequence defines the 5' splice site of a typical eukaryotic nuclear intron?
(A) An invariant AG
(B) An invariant GU
(C) A conserved TATA sequence
(D) A branch point adenosine

2012

**70.** During the first catalytic step of pre-mRNA splicing, which chemical group performs the nucleophilic attack on the 5' splice site?
(A) The 2'-OH of the branch point adenosine.
(B) The 3'-OH of the preceding exon sequence.
(C) The 5'-phosphate of the intron sequence.
(D) The 2'-OH of the 5' cap on the transcript.

2012

**71.** What specific intermediate structure is formed during the typical spliceosome-mediated splicing reaction?
(A) A lariat structure
(B) A hairpin loop
(C) A linear concatemeric RNA
(D) A double-stranded RNA hybrid

2011

**72.** Which functional role do catalytic RNAs (ribozymes) play in group I intron splicing?
(A) They require ATP hydrolysis for catalytic activity in all cases.
(B) They utilize a branch point adenosine for the first step reaction.
(C) They assemble into large ribonucleoprotein complexes for splicing.
(D) They catalyze excision without the need for a spliceosome.

2021

**73.** What is the most profound biological impact of alternative splicing in eukaryotes?
(A) Generating protein isoforms from a single gene locus.
(B) Complete elimination of introns from the entire genome.
(C) Prevention of viral RNA integration into host chromosomes.
(D) Rapid turnover of all messenger RNAs within the cytoplasm.

2024

**74.** Which process most likely contributes to tissue-specific regulation of protein diversity?
(A) Uniform RNA degradation.
(B) Universal translation initiation.
(C) Tissue-specific alternative splicing.
(D) Constant poly-A tail shortening.

2007

**75.** Given below are four statements concerning the properties of the genetic code:
I. The code is highly degenerate.
II. The code is strictly overlapping.
III. The code is nearly universal.
IV. The code is read without punctuation.
Which of the above statement(s) is/are INCORRECT?
(A) I and III
(B) II only
(C) III and IV
(D) II and IV

2009

**76.** The concept of degeneracy in the genetic code refers to which specific feature?
(A) Multiple codons can code for the same amino acid.
(B) A single codon can code for multiple amino acids.
(C) The code evolves rapidly and degrades over time.
(D) The third base of every codon is always unassigned.

2014

**77.** Which hypothesis mathematically explains how a single tRNA can recognize multiple codons?
(A) The central dogma
(B) The wobble hypothesis
(C) The endosymbiotic hypothesis
(D) The adapter hypothesis

2001

**78.** According to the wobble hypothesis, which base position of the anticodon allows for non-standard base pairing?
(A) The first position (5' end) of the anticodon
(B) The second position of the anticodon
(C) The third position (3' end) of the anticodon
(D) The position adjacent to the CCA tail

2017

**79.** The near-universality of the genetic code implies which fundamental evolutionary principle?
(A) All organisms possess identical genome sequences throughout life.
(B) Prokaryotes and eukaryotes utilize identical ribosomal structures.
(C) Viral genomes do not rely on standard codons for protein synthesis.
(D) The code evolved early and was heavily conserved.

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2002

**80.** Identify the INCORRECT pair regarding specific codon assignments.
(A) AUG – Methionine / Start
(B) UAA – Stop
(C) UGA – Leucine
(D) UGG – Tryptophan

2002
**81.** Which subunit of the prokaryotic ribosome is responsible for decoding the mRNA template?
(A) 40S subunit
(B) 50S subunit
(C) 60S subunit
(D) 30S subunit

2011
**82.** In the typical eukaryotic ribosome (80S), what are the sedimentation coefficients of the constituent subunits?
(A) 50S and 30S
(B) 60S and 40S
(C) 40S and 30S
(D) 70S and 20S

2013
**83.** The secondary structure of transfer RNA (tRNA) is universally described as resembling which shape?
(A) A double helix
(B) A cloverleaf
(C) An L-shape
(D) A linear stem

2001
**84.** Which functional region of the tRNA molecule covalently attaches to the specific amino acid?
(A) The D loop
(B) The 3' CCA acceptor stem
(C) The T-psi-C loop
(D) The anticodon loop

2008
**85.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Aminoacyl-tRNA synthetases are highly specific enzymes.
**Reason (R):** They must accurately pair a specific amino acid with its corresponding tRNA to maintain the fidelity of translation.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2019
**86.** What is the fundamental mechanism utilized by some aminoacyl-tRNA synthetases to correct mischarging errors?
(A) Reverse transcription of the tRNA
(B) A proofreading editing site that hydrolyzes incorrectly paired amino acids
(C) Degradation of the entire ribosome
(D) Splicing of the tRNA anticodon

2012
**87.** During prokaryotic translation initiation, the Shine-Dalgarno sequence base-pairs with which ribosomal component?
(A) The 16S rRNA of the 30S subunit
(B) The 5S rRNA
(C) The 23S rRNA of the 50S subunit
(D) The initiator tRNA

2023
**88.** Which initial specific factor binds to the eukaryotic mRNA 5' cap during translation initiation?
(A) $eIF_2$
(B) EF-Tu
(C) eIF4E
(D) $RF_1$

2008
**89.** In the elongating ribosome, an incoming aminoacyl-tRNA first enters which specific site?
(A) The E (Exit) site
(B) The A (Aminoacyl) site
(C) The P (Peptidyl) site
(D) The Shine-Dalgarno site

2021
**90.** Which enzymatic activity is directly responsible for peptide bond formation during translation?
(A) Aminoacyl-tRNA synthetase
(B) Peptidyl transferase
(C) RNA polymerase
(D) Signal peptidase

2003
**91.** Current structural evidence demonstrates that peptidyl transferase activity is a function of:
(A) A specific ribosomal protein in the large subunit
(B) The 23S or 28S ribosomal RNA
(C) The mRNA transcript itself
(D) Elongation factor G

2010
**92.** What occurs during the translocation step of translation elongation?
(A) The ribosome shifts one codon toward the 3' end of the mRNA.
(B) The empty tRNA is moved from the P site to the A site.
(C) The mRNA is cleaved by a ribonuclease.
(D) The release factor binds to the stop codon.

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2013
**93.** Which factor recognizes a stop codon in the A site during translation termination in prokaryotes?
(A) A stop tRNA
(B) Elongation factor Tu
(C) Release factor ($RF_1$ or $RF_2$)
(D) Ribosome recycling factor

2022
**94.** What is the direct outcome of release factor binding to a stop codon?
(A) Addition of a final poly-A tail to the protein
(B) Covalent linkage of the ribosome subunits
(C) Immediate destruction of the mRNA
(D) Hydrolysis of the ester bond linking the polypeptide to the tRNA in the P site

2003
**95.** Match the post-translational modification in List I with its typical functional consequence in List II.
List I: (Post-Translational Modification)
P. Phosphorylation
Q. Ubiquitination
R. Glycosylation
S. Acetylation
List II: (Functional Consequence)
1. Targets protein for proteasomal degradation
2. Alters protein activity or interaction via kinase action
3. Affects chromatin structure by neutralizing lysine charge
4. Modulates protein folding, stability, and cell-surface recognition
(A) P-2, Q-1, R-4, S-3
(B) P-1, Q-2, R-3, S-4
(C) P-3, Q-4, R-1, S-2
(D) P-4, Q-3, R-2, S-1

2024
**96.** Which class of enzymes specifically removes phosphate groups from proteins?
(A) Kinases
(B) Phosphatases
(C) Ligases
(D) Transferases

2003
**97.** The attachment of ubiquitin to a target protein typically occurs on which amino acid residue?
(A) Serine
(B) Tyrosine
(C) Lysine
(D) Methionine

2002
**98.** Which biochemical consequence is most consistently associated with the hyperacetylation of core histone proteins?
(A) Transcriptional activation
(B) Transcriptional repression
(C) Immediate protein degradation
(D) Inhibition of RNA splicing

2025
**99.** In Electrophoretic Mobility Shift Assay (EMSA), a protein-DNA complex migrates differently than free DNA. What is the physical basis for this shift?
(A) The complex is larger and has a different charge, thus migrating slower.
(B) The complex is degraded, moving faster.
(C) The protein digests the DNA, creating smaller, faster fragments.
(D) The DNA becomes highly negatively charged and migrates instantly to the pole.

2019
**100.** DNase I footprinting is designed to identify which specific molecular characteristic?
(A) The exact size of an mRNA transcript
(B) The specific DNA sequence bound by a protein
(C) The rate of translation elongation
(D) The tertiary structure of a transfer RNA

2019
**101.** Which fundamental principle underlies the DNase I footprinting technique?
(A) DNase I selectively cuts only at specific promoter consensus sequences.
(B) DNase I degrades all RNA transcripts present in the nucleus.
(C) Bound proteins actively stimulate DNase I to cut immediately adjacent DNA.
(D) DNase I randomly cleaves unprotected DNA but cannot cut where a protein is tightly bound.

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2016
**102.** Chromatin immunoprecipitation (ChIP) is primarily used to analyze which phenomenon in vivo?
(A) RNA-RNA interactions
(B) The association of specific proteins with specific genomic regions
(C) The subcellular localization of mitochondria
(D) The precise mass of a translated protein

2009

**103.** In a standard ChIP assay, what is typically the first step performed on live cells?
(A) Immediate extraction of bare DNA
(B) Crosslinking of proteins to DNA using formaldehyde
(C) Digestion of the cell wall with lysozyme
(D) Sequencing of the entire genome

2023

**104.** The yeast two-hybrid system is an analytical technique used primarily to investigate:
(A) Protein-protein interactions
(B) DNA-RNA hybridization kinetics
(C) The speed of ribosomal translocation
(D) The extent of DNA methylation

2015

**105.** Given below are three statements regarding the yeast two-hybrid system:
I. It relies on the modular nature of eukaryotic transcription factors (e.g., GAL4).
II. The "bait" protein is fused to a DNA-binding domain.
III. The "prey" protein is fused to an activation domain.
Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I, II and III

2022

**106.** In prokaryotic gene regulation, what defines a "positive control" system?
(A) Transcription requires the binding of an activator protein to the promoter region.
(B) The regulatory protein inhibits transcription when bound.
(C) The operon is permanently switched off.
(D) The system relies exclusively on RNA interference.

2008

**107.** Which statement accurately describes a repressible operon system?
(A) It is usually off and must be turned on by a small molecule.
(B) It relies entirely on positive activation by cAMP.
(C) It is usually on and is turned off when a specific corepressor is present.
(D) It functions without any repressor protein.

2001

**108.** The Lac operon of E. coli is considered which type of regulatory system?
(A) Inducible
(B) Repressible
(C) Constitutive
(D) Attenuated

2002

**109.** What is the functional role of the LacI protein in the regulation of the Lac operon?
(A) It acts as an inducer.
(B) It transports lactose into the cell.
(C) It catalyzes the breakdown of lactose into glucose and galactose.
(D) It functions as an allosteric repressor binding to the operator.

2021

**110.** How does the presence of allolactose (or IPTG) affect the Lac repressor?
(A) It binds the repressor, inducing a conformational change that prevents operator binding.
(B) It covalently modifies the repressor, causing its rapid degradation.
(C) It assists the repressor in binding tighter to the promoter.
(D) It directly activates RNA polymerase.

2001

**111.** Which phenomenon explains the preferential use of glucose over lactose by E. coli?
(A) Transcriptional attenuation
(B) Feedback inhibition of the repressor
(C) Ribosomal frameshifting
(D) Catabolite repression

2004

**112.** In the mechanism of catabolite repression, high levels of glucose lead to which intracellular condition?
(A) Low levels of cyclic AMP (cAMP)
(B) High levels of cyclic AMP (cAMP)
(C) Immediate phosphorylation of the LacI repressor
(D) Hyperactivation of the CAP protein

2005

**113.** What is required for the CAP (Catabolite Activator Protein) to bind the Lac promoter and stimulate transcription?
(A) High ATP concentrations
(B) The presence of bound repressor
(C) Binding of cAMP to the CAP protein
(D) The complete absence of lactose

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2015

**114.** The Trp operon in E. coli exemplifies which class of regulatory control?
(A) Inducible negative control
(B) Repressible negative control
(C) Constitutive positive control
(D) RNAi-mediated silencing

2019

**115.** Given below are four statements regarding transcriptional attenuation in the Trp operon:
I. It relies on the tight coupling of transcription and translation in prokaryotes.
II. It involves the formation of alternative secondary structures in the leader mRNA.
III. It functions completely independently of the Trp repressor protein.
IV. High tryptophan levels cause the formation of a terminator hairpin.
Which of the above statement(s) is/are INCORRECT?
(A) I and II
(B) III only
(C) I and IV
(D) II and III

2014

**116.** During Trp operon attenuation, what specific event occurs if tryptophan levels are exceptionally low?
(A) The ribosome stalls at tandem Trp codons, allowing an anti-terminator hairpin to form.
(B) The ribosome speeds up, causing immediate termination.
(C) The RNA polymerase dissociates before transcribing the leader peptide.
(D) The Trp repressor prepares permanently to the operator.

2019

**117.** Which mechanistic factor is critical for the success of attenuation as a regulatory strategy?
(A) The simultaneous occurrence of transcription and translation
(B) The spatial separation of the nucleus and cytoplasm
(C) The presence of a poly-A tail
(D) The action of the spliceosome

2002

**118.** Eukaryotic DNA methylation predominantly occurs on which nucleotide base?
(A) Adenine
(B) Guanine
(C) Cytosine
(D) Thymine

2006

**119.** DNA methylation at CpG islands situated in eukaryotic promoter regions is generally associated with:
(A) Transcriptional activation
(B) High rates of spontaneous mutation to adenine
(C) Transcriptional silencing
(D) Enhanced recruitment of RNA polymerase I

2002

**120.** Which term refers to the hypothesis that specific combinations of post-translational modifications on histone tails dictate distinct functional outcomes for chromatin?
(A) The histone code
(B) The wobble hypothesis
(C) The endosymbiotic theory
(D) The replicon model

2012

**121.** ATP-dependent chromatin remodeling complexes perform which specific biochemical function?
(A) Covalently modify histone tails with acetyl groups
(B) Hydrolyze ATP to slide, eject, or restructure nucleosomes
(C) Directly methylate DNA at CpG dinucleotides
(D) Cleave pre-mRNA to initiate splicing

2025

**122.** Which molecular entity is central to the mechanism of RNA interference (RNAi)?
(A) Small double-stranded RNA molecules
(B) Long single-stranded DNA
(C) Catalytic proteins completely devoid of nucleic acids
(D) Ribosomal RNA subunits

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2014

**123.** The enzyme responsible for cleaving long double-stranded RNA or pre-miRNA into short fragments (~21-23 nt) during RNAi is known as:
(A) Drosha
(B) Dicer
(C) Argonaute
(D) Slicer

2013

**124.** What is the primary functional core of the RNA-induced silencing complex (RISC)?
(A) RNA polymerase III
(B) The Argonaute protein
(C) The spliceosome
(D) DNA ligase

2007

**144.** Spliceosome assembly occurs sequentially. Which snRNP initially recognizes and binds the 5' splice site?
(A) $U_1$
(B) $U_2$
(C) $U_4$
(D) $U_6$

2004

**145.** Given below are five statements concerning the RNA splicing machinery:
I. $U_1$ snRNP binds the 5' splice site.
II. $U_2$ snRNP binds the branch point sequence.
III. The $U_4/U_6$-$U_5$ tri-snRNP complex enters the spliceosome.
IV. $U_1$ and $U_4$ are expelled prior to the first transesterification.
V. The catalytic core of the active spliceosome is formed primarily by snRNAs.
Which of the above statements are correct?
(A) I, II, III and V
(B) I, II, IV and V
(C) II, III, IV and V
(D) I, II, III, IV and V

2016

**146.** Which structural feature ensures that an aminoacyl-tRNA synthetase does not charge a tRNA with the wrong amino acid?
(A) The precise fit of the amino acid in the activation site and a separate editing site
(B) The poly-A tail of the mRNA
(C) The interaction with the ribosomal large subunit
(D) The methylation of the anticodon

2021

**147.** During translation elongation in eukaryotes, which factor is responsible for delivering the aminoacyl-tRNA to the A site?
(A) $eEF_1A$ (equivalent to prokaryotic EF-Tu)
(B) $eEF_2$ (equivalent to prokaryotic EF-G)
(C) eIF4G
(D) $eRF_1$

2015

**148.** Which energetic molecule is hydrolyzed during the delivery of an aminoacyl-tRNA to the ribosome?
(A) GTP
(B) ATP
(C) CTP
(D) UTP

2016

**149.** A mutation in the gene encoding $eRF_1$ (eukaryotic Release Factor 1) that eliminates its function would immediately lead to:
(A) Prevention of ribosome assembly
(B) Stalling of ribosomes at stop codons without chain release
(C) Random frameshifting throughout elongation
(D) Premature termination of all transcripts

2006

**150.** Which post-translational modification is explicitly characterized by the addition of complex carbohydrate chains to specific asparagine residues?
(A) O-linked glycosylation
(B) N-linked glycosylation
(C) S-palmitoylation
(D) N-terminal acetylation

2005

**151.** Where does N-linked glycosylation of newly synthesized proteins predominantly initiate within a eukaryotic cell?
(A) The nucleoplasm
(B) The Golgi apparatus
(C) The rough endoplasmic reticulum
(D) The lysosome

2002

**152.** Which mechanism best illustrates "negative control" in the regulation of the lac operon?
(A) The suppression of transcription by the Lac repressor binding the operator
(B) The binding of RNA polymerase to the promoter
(C) The activation of transcription by the CAP-cAMP complex
(D) The induction of the system by allolactose

2022

**153.** A mutation in the lac operator (lacOc) that prevents repressor binding results in which phenotype?
(A) Constitutive expression of the lac operon
(B) Permanent repression of the operon system
(C) Lethality due to high lactose cell toxicity
(D) Inability to synthesize intracellular cAMP

2003

**154.** What is the specific structural result of chromatin remodeling complexes pushing nucleosomes away from a promoter?
(A) The promoter becomes heavily methylated.
(B) Transcription factors and RNA polymerase can access the DNA.

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(A) They require exactly zero computational power.
(B) They are highly effective for creating probabilistic profiles of protein families and identifying remote homologs.
(C) They instantly assemble full genomes from short reads.
(D) They physically isolate DNA fragments from agarose gels.

2004

**135.** Match the molecular analysis technique/concept in List I with its description in List II.
List I: (Analysis Technique/Concept)
P. In silico identification
Q. Homology alignment
R. ChIP-seq
S. Transcriptomics
List II: (Description)
1. Profiling all expressed RNAs
2. Mapping protein binding sites across the genome
3. Computational discovery of novel genes
4. Comparing sequences for evolutionary conservation
(A) P-3, Q-4, R-2, S-1
(B) P-4, Q-3, R-1, S-2
(C) P-1, Q-2, R-3, S-4
(D) P-2, Q-1, R-4, S-3

2011

**136.** Which term describes genes in different species that evolved from a common ancestral gene via speciation?
(A) Paralogs
(B) Orthologs
(C) Analogs
(D) Xenologs

2013

**137.** How do paralogous genes typically arise within a genome?
(A) Through Horizontal gene transfer
(B) Through speciation events
(C) Through gene duplication events
(D) Through viral integration

2024

**138.** The identification of novel alleles of agronomically important traits heavily utilizes which bioinformatic resource?
(A) Single Nucleotide Polymorphism (SNP) databases and GWAS
(B) Electron microscopy databases
(C) Spectrophotometry logs
(D) Purely phenotypic field records

2001

**139.** Which method provides the highest resolution for determining the global complexity of a newly discovered plant genome?
(A) Whole-genome Next Generation Sequencing (NGS)
(B) Cot curve analysis
(C) Flow cytometry
(D) Karyotyping

2008

**140.** Which factor introduces the most significant challenge when computationally annotating a large eukaryotic genome?
(A) The presence of a poly-A tail on mRNAs
(B) The lack of any recognizable start codons
(C) The massive abundance of highly repetitive DNA and long introns
(D) The universal absence of promoter sequences

2004

**141.** The specific identification of an origin of replication (ARS) in yeast relies on which characteristic?
(A) High GC content
(B) High transcriptional activity
(C) An AT-rich consensus sequence that binds the ORC
(D) The presence of heavily methylated cytosines

2002

**142.** A genomic region that lacks a TATA box but initiates transcription from a focused start point typically relies on which element?
(A) The Initiator (Inr) element
(B) An enhancer located millions of base pairs away
(C) The Shine-Dalgarno sequence
(D) A telomere

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2009

**143.** Which statement accurately reflects the role of the CTD (Carboxy-Terminal Domain) of RNA polymerase II?
(A) It binds specific operator sequences.
(B) Its phosphorylation state coordinates transcription elongation and RNA processing events.
(C) It acts as a helicase to unwind the DNA.
(D) It directly synthesizes the poly-A tail.

cap and 3' poly-A tail
(B) The presence of a promoter sequence
(C) The inclusion of all transcribed introns
(D) The permanent attachment to RNA polymerase II

2005
**204.** By which mechanism does the small nuclear RNA $U_1$ recognize the 5' splice site?
(A) Complementary base pairing with the 5' splice site consensus sequence
(B) Protein-protein interaction with RNA polymerase
(C) Covalent bonding to the DNA template
(D) Nonspecific electrostatic attraction

2022
**205.** Given below are four events of translation initiation in E. coli:
I. The 50S subunit binds, hydrolyzing GTP and releasing IFs.
II. $IF_1$, $IF_2$-GTP, and IF3 bind the 30S subunit.
III. The initiator fMet-tRNA binds the AUG codon at the P site.
IV. The 30S subunit binds the mRNA via the Shine-Dalgarno sequence.
Which of the following represents the correct biological sequence?
(A) II, IV, III, I
(B) IV, II, III, I
(C) II, III, IV, I
(D) III, IV, II, I

2025
**206.** Which structural transition defines the shift from a closed to an open complex during prokaryotic transcription?
(A) The localized melting of approximately 14 base pairs of DNA around the start site
(B) The release of the sigma factor
(C) The formation of the first phosphodiester bond
(D) The binding of the rho protein

2008
**207.** What constitutes the primary mechanism of action for small interfering RNAs (siRNAs) in a eukaryotic cell?
(A) They bind to specific mRNAs and guide the RISC complex to cleave them.
(B) They act as primers for DNA replication.
(C) They upregulate translation by binding the 5' cap.
(D) They directly methylate genomic DNA at specific loci.

2010
**208.** During RNAi, the "passenger strand" of the initial double-stranded RNA intermediate is:
(A) Converted into a transfer RNA
(B) Exported to the extracellular space
(C) Degraded, leaving the "guide strand" active within RISC
(D) Loaded into the Argonaute protein to guide cleavage

2004
**209.** What is the fundamental difference between an enhancer and a promoter?
(A) Enhancers are proteins; promoters are DNA.
(B) Promoters act exclusively on RNA polymerase I; enhancers act exclusively on polymerase III.
(C) Promoters are found only in prokaryotes; enhancers only in eukaryotes.
(D) Promoters determine the exact start site and basal direction of transcription; enhancers modulate the rate independent of position or orientation.

2012
**210.** The biological consequence of extensive DNA methylation at a specific gene locus is most likely:
(A) A massive increase in transcription factor binding
(B) The formation of highly active euchromatin
(C) The recruitment of methyl-CpG-binding domain (MBD) proteins and subsequent gene silencing
(D) Rapid recruitment of the spliceosome

2015
**211.** Which modification is universally viewed as a hallmark of transcriptionally active chromatin (euchromatin)?
(A) High levels of histone acetylation
(B) High levels of DNA methylation
(C) Absence of any histone proteins
(D) High levels of $H_3K27$ trimethylation

2012
**212.** What primary structural feature makes the DNA major groove the preferred binding site for most sequence-specific transcription factors?
(A) It is narrower, making it easier for proteins to grasp.
(B) It is entirely devoid of electronegative charges.
(C) It actively repels water molecules, increasing binding affinity.
(D) It presents a distinct pattern of hydrogen

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(C) The promoter region becomes sterically inaccessible.
(D) The DNA double helix permanently unwinds into single strands.

2024
**155.** Given below are four steps of an Electrophoretic Mobility Shift Assay (EMSA):
I. Electrophoresis on a non-denaturing polyacrylamide gel
II. Incubation of labeled DNA with the protein extract
III. Detection of the labeled DNA fragments
IV. Preparation of a radioactively or fluorescently labeled DNA probe
Which of the following represents the correct sequential order?
(A) IV, II, I, III
(B) IV, I, II, III
(C) II, IV, I, III
(D) I, IV, II, III

2009
**156.** In analyzing a typical eukaryotic transcription unit, which feature provides the most definitive evidence of a spliced transcript?
(A) Alignment of cDNA to the genome showing continuous gaps corresponding to introns
(B) Presence of a poly-A sequence at the 3' end
(C) Presence of a 5' cap
(D) Identification of an open reading frame

2009
**157.** Which characteristic is least relevant to the structural classification of a transcription factor?
(A) The presence of a zinc finger motif
(B) The length of its poly-A tail
(C) The existence of a basic helix-loop-helix domain
(D) The sequence of its DNA-binding domain

2006
**158.** The phenomenon where a single gene produces distinct functional mRNAs in the brain versus the liver is best explained by:
(A) Alternative splicing
(B) Differential gene loss
(C) Somatic recombination
(D) Variable DNA methylation of the structural exons

2016
**159.** What is the defining characteristic of a strictly constitutive gene?
(A) It is transcribed only under extreme environmental stress conditions.
(B) It requires a continuous supply of inducer molecules for activity.
(C) It is expressed at a constant level under all conditions.
(D) It is regulated by the transcriptional attenuation mechanism.

2022
**160.** Which molecule serves as the direct co-repressor for the Trp operon?
(A) Tryptophan
(B) The Trp repressor protein
(C) Attenuator RNA
(D) Cyclic AMP

2019
**161.** An E. coli cell experiences a sudden depletion of glucose and an abundance of lactose. What is the immediate regulatory response at the lac operon?
(A) Repressor dissociates; CAP binds with cAMP to activate transcription
(B) Repressor remains bound; CAP dissociates
(C) Both Repressor and CAP bind simultaneously
(D) The operon undergoes immediate attenuation

2016
**162.** How does the presence of an insulator element placed between an enhancer and a promoter alter transcriptional dynamics?
(A) It amplifies the enhancer's signal.
(B) It prevents the enhancer from activating that specific promoter.
(C) It converts the enhancer into a repressor.
(D) It randomly initiates ectopic transcription.

2005
**163.** The addition of a 7-methylguanosine cap to pre-mRNA is catalyzed by enzymes associated with which molecular structure?
(A) The spliceosome
(B) The large ribosomal subunit
(C) The phosphorylated C-terminal domain of RNA polymerase II
(D) The nuclear pore complex

2012
**164.** What happens to the lariat intron immediately after its release from the spliceosome?
(A) It acts as an independent small nuclear RNA.
(B) It is exported to the cytoplasm for translation.
(C) It is debranched and rapidly degraded by nuclear ribonucleases.
(D) It covalently binds to the 3' end of the

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2014

**125.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).

**Assertion (A):** MicroRNAs (miRNAs) can inhibit translation without perfectly pairing with the target mRNA.

**Reason (R):** Perfect sequence complementarity is an absolute requirement for Argonaute-mediated transcript cleavage, whereas partial pairing leads to translational repression.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2022

**126.** Which feature largely distinguishes siRNAs from miRNAs in their typical cellular biogenesis?

(A) siRNAs are usually derived from exogenous long dsRNA, whereas miRNAs are encoded by endogenous cellular genes.
(B) siRNAs are processed in the nucleus, while miRNAs bypass the nucleus entirely.
(C) siRNAs are single-stranded upon formation, while miRNAs remain permanently double-stranded.
(D) siRNAs require ribosomes for their biogenesis.

2006

**127.** Which process best exemplifies post-transcriptional gene regulation?

(A) Alternative splicing and mRNA stability control
(B) Histone acetylation
(C) Promoter methylation
(D) Chromatin remodeling

2024

**128.** How does the phosphorylation of eukaryotic initiation factor 2 ($eIF_2$) globally affect cellular translation?

(A) It massive upregulates translation by increasing ribosome assembly.
(B) It causes a general inhibition of translation initiation by blocking nucleotide exchange.
(C) It selectively activates the translation of all housekeeping genes.
(D) It prevents the binding of the poly-A binding protein.

2021

**129.** What is the functional impact of upstream open reading frames (uORFs) located in the 5' untranslated region of a eukaryotic mRNA?

(A) They frequently repress the translation of the downstream main coding sequence.
(B) They consistently act as powerful enhancers of main ORF translation.
(C) They serve as targets for RNA polymerase III.
(D) They trigger immediate mRNA decapping in all contexts.

2004

**130.** The stability of a eukaryotic mRNA transcript is most heavily dependent upon the integrity of which two structures?

(A) The TATA box and the Inr element
(B) The 5' cap and the 3' poly-A tail
(C) The promoter and the terminator
(D) The D loop and the anticodon

2011

**131.** Which intracellular pathway is responsible for degrading mRNAs containing premature stop codons?

(A) Nonsense-mediated decay (NMD)
(B) Ubiquitin-proteasome system
(C) Lysosomal autophagy
(D) Apoptosis

2004

**132.** In the context of gene mining strategies, what does "homology-based cloning" primarily rely upon?

(A) Using conserved sequence motifs from known genes to design primers or probes to isolate related genes
(B) Generating entirely random DNA sequences
(C) Identifying genes exclusively through structural X-ray crystallography
(D) Directly sequencing unknown proteins

NQDIA

2016

**133.** Which computational algorithm is the fundamental standard for local sequence alignment in bioinformatics?

(A) BLAST (Basic Local Alignment Search Tool)
(B) Hidden Markov Models (HMM)
(C) Fast Fourier Transform
(D) Monte Carlo simulation

2003

**134.** What is the specific advantage of using Hidden Markov Models (HMM) in sequence analysis?

(C) N-linked glycosylation
(D) 3' cleavage and polyadenylation

2008

**175.** Match the translational component in List I with its corresponding function in List II.
List I: (Translational Component)
P. eIF4E
Q. EF-G (or $eEF_2$)
R. $eRF_1$
S. Peptidyl transferase
List II: (Corresponding Function)
1. Catalyzes peptide bond formation
2. Binds the 5' cap of mRNA
3. Recognizes stop codons
4. Mediates ribosomal translocation
(A) P-2, Q-4, R-3, S-1
(B) P-1, Q-2, R-4, S-3
(C) P-3, Q-1, R-2, S-4
(D) P-4, Q-3, R-1, S-2

2023

**176.** Which condition directly triggers the attenuation mechanism to terminate transcription in the Trp operon?
(A) High intracellular levels of charged tRNA-Trp
(B) Complete absence of tryptophan in the medium
(C) High levels of intracellular glucose
(D) High intracellular levels of uncharged tRNA-Trp

2017

**177.** The discovery of catalytic RNA challenged which long-held biochemical dogma?
(A) DNA is the genetic material.
(B) All biological catalysts (enzymes) are proteins.
(C) The genetic code is universal.
(D) Transcription precedes translation.

2015

**178.** What is the primary analytical goal of the ChIP-seq technique?
(A) To identify all genomic binding sites for a transcription factor or modified histone in vivo
(B) To sequence an entire genome de novo
(C) To measure the precise halF-life of an mRNA
(D) To detect single nucleotide polymorphisms in a population

2012

**179.** In silico gene mining relies heavily on which foundational assumption?
(A) All organisms possess identical gene sequences.
(B) Genes with similar sequences (homologs) likely share similar evolutionary origins and functions.
(C) Non-coding regions are strictly conserved across all taxa.
(D) Only perfectly identical sequences can perform the same function.

2014

**180.** Which database search algorithm uses a position-specific scoring matrix to detect distant evolutionary relationships?
(A) Standard Megablast
(B) PSI-BLAST
(C) Clustal Omega
(D) Needleman-Wunsch

2004

**181.** The 16S rRNA plays a critical role in prokaryotic translation. Which of the following is NOT one of its functions?
(A) Providing structural integrity to the 30S subunit
(B) Base-pairing with the Shine-Dalgarno sequence
(C) Stabilizing correct codon-anticodon pairing
(D) Acting as the primary peptidyl transferase

2008

**182.** A nonsense mutation in the middle of a structural gene results in:
(A) A silent substitution with no functional consequence
(B) The production of a truncated polypeptide
(C) Enhanced continuous translation into the 3' UTR
(D) A complete failure of transcription initiation

2007

**183.** Identify the INCORRECT statement regarding eukaryotic mRNA stability.
(A) Shortening of the poly-A tail typically initiates mRNA degradation.
(B) The 5' cap protects the transcript from 3' to 5' exonucleases.
(C) AU-rich elements (AREs) in the 3' UTR generally promote rapid mRNA decay.
(D) Decapping enzymes act only after significant poly-A tail removal in the default decay pathway.

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mRNA.

2004

**165.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).

**Assertion (A):** The genetic code is degenerate, meaning most amino acids are specified by more than one codon.

**Reason (R):** The wobble position allows a single tRNA anticodon to base pair with multiple synonymous codons, typically varying at the third nucleotide position.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2003

**166.** A critical difference between prokaryotic and eukaryotic translation initiation is:

(A) Eukaryotic initiation generally relies on ribosomal scanning from the 5' cap, while prokaryotic relies on internal Shine-Dalgarno binding.
(B) Prokaryotic translation requires a 5' cap.
(C) Eukaryotic ribosomes initiate at completely random codons.
(D) Eukaryotic initiation factor 2 ($eIF_2$) is totally independent of GTP.

2010

**167.** Which of the following best describes the "histone code" hypothesis?

(A) Histones encode unique structural proteins.
(B) Covalent modifications of histone tails serve as docking sites for chromatin-modifying effector proteins.
(C) Histone mRNA sequences uniquely lack stop codons.
(D) Histone proteins physically replace RNA polymerase during repression.

2025

**168.** Which specific modification is commonly associated with transcriptionally inactive heterochromatin?

(A) High levels of histone acetylation
(B) Trimethylation of Histone $H_3$ at Lysine 9 ($H_3K_9me_3$)
(C) Hypomethylation of DNA at CpG islands
(D) Extensive phosphorylation of RNA polymerase II

2012

**169.** What functional domain is essential for a eukaryotic transcription factor to recruit chromatin remodeling complexes?

(A) The transactivation domain
(B) The DNA-binding domain
(C) The nuclear export signal
(D) The zinc finger motif

2004

**170.** Which mechanism is least likely to result in post-transcriptional gene silencing?

(A) Binding of miRNAs to the 3' UTR of mRNA
(B) Targeted cleavage of mRNA by the RISC complex
(C) Extensive methylation of the core promoter
(D) Nonsense-mediated mRNA decay

2007

**171.** A primary transcript is found to be 5000 nucleotides long, but the mature mRNA is only 1200 nucleotides. This discrepancy is overwhelmingly due to:

(A) 5' capping
(B) RNA splicing
(C) Polyadenylation
(D) Proteolytic cleavage

NODIA

2007

**172.** The presence of overlapping genes in a viral genome implies which specific molecular constraint?

(A) A single mutation could simultaneously alter multiple distinct viral proteins.
(B) The virus has an exceptionally large genome.
(C) The viral RNA polymerase is highly inaccurate.
(D) The virus does not rely on host ribosomes.

2006

**173.** What is the fundamental difference between the "muton" and the "cistron" as classically defined by Benzer?

(A) The muton is the smallest unit of mutation (a single base pair), while the cistron is the unit of function (a gene).
(B) The muton is larger than the cistron.
(C) The muton applies only to RNA; the cistron applies only to DNA.
(D) There is no difference; they are synonymous.

2015

**174.** Which process does not typically occur cotranscriptionally in eukaryotes?

(A) 5' capping
(B) Splicing of some introns

2008

**194.** Which structural domain allows transcription factors to form dimeric complexes prior to DNA binding?
(A) The leucine zipper
(B) The zinc finger exclusively
(C) The TATA-binding domain
(D) The core promoter Inr element

2003

**195.** Given below are four general transcription factors involved in pre-initiation complex formation:
I. TFIIE and TFIIH
II. TFIID (via TBP)
III. RNA Polymerase II and TFIIF
IV. TFIIA and TFIIB
Which of the following represents the correct sequential order of assembly?
(A) II, IV, III, I
(B) II, I, IV, III
(C) IV, II, I, III
(D) III, IV, II, I

2008

**196.** The phosphorylation of the RNA polymerase II CTD at Serine 5 is primarily associated with which transcription phase?
(A) Promoter escape and initiation of 5' capping
(B) Promoter binding
(C) Termination of transcription
(D) Recruitment of polyadenylation factors

2024

**197.** Which analytical technique would be most appropriate to identify the specific nucleotide sequence where an unknown transcription factor binds a promoter?
(A) Northern blotting
(B) Western blotting
(C) Mass spectrometry
(D) DNase I footprinting

2022

**198.** In the yeast two-hybrid system, a positive protein-protein interaction is visually or selectively identified by:
(A) The transcriptional activation of a reporter gene (e.g., LacZ or $HIS_3$)
(B) The massive aggregation of the yeast cells
(C) The rapid death of the yeast cells
(D) The physical isolation of a lariat structure

2024

**199.** What fundamental property of moderately repetitive DNA sequences, such as LINEs, distinguishes them from highly repetitive satellite DNA?
(A) They are generally longer, interspersed throughout the genome, and may encode reverse transcriptase.
(B) They are completely devoid of any open reading frames.
(C) They are exclusively localized to the centromeres.
(D) They consist exclusively of short 2-5 bp tandem repeats.

2011

**200.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Alternative splicing increases the coding capacity of a eukaryotic genome.
**Reason (R):** It allows a single pre-mRNA to be processed into multiple distinct mature mRNAs, producing different protein isoforms.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2008

**201.** Which RNA sequence directs the precise cleavage of a eukaryotic transcript prior to polyadenylation?
(A) The consensus sequence AAUAAA
(B) The TATA box
(C) The Shine-Dalgarno sequence
(D) The Kozak consensus sequence

2022

**202.** If a researcher identifies a genomic sequence characterized by extreme sequence conservation across divergent species, what is the most likely inference?
(A) The sequence is non-functional "junk" DNA.
(B) The sequence is under strong negative (purifying) selection due to essential functionality.
(C) The sequence represents a rapidly evolving pseudogene.
(D) The sequence is entirely composed of SINE elements.

2005

**203.** Which feature distinguishes a mature eukaryotic mRNA from its primary pre-mRNA transcript?
(A) The absence of introns and presence of a 5'

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2025

**184.** In the context of microRNA function, what determines whether a target mRNA is cleaved or simply translationally repressed?
(A) The concentration of Dicer in the cytoplasm
(B) The degree of base-pairing complementarity between the miRNA and the mRNA
(C) The presence of a 5' cap on the miRNA
(D) The precise location of the gene in the nucleus

2001

**185.** Given below are five statements regarding post-translational modifications:
I. Phosphorylation is typically a reversible process.
II. Ubiquitination invariably activates the target protein.
III. Glycosylation frequently aids in correct protein folding.
IV. Acetylation of histones neutralizes the positive charge of lysine.
V. Kinases add phosphate groups, while phosphatases remove them.
Which of the above statement(s) is/are INCORRECT?
(A) I and II
(B) II only
(C) III and V
(D) IV and V

2004

**186.** During the formation of the eukaryotic 43S pre-initiation complex, which component brings the initiator tRNA to the small ribosomal subunit?
(A) $eIF_2$ complexed with GTP
(B) eIF4E
(C) The poly-A binding protein (PABP)
(D) The large 60S subunit

2005

**187.** What mechanism allows for the coordinated regulation of multiple genes scattered across different chromosomes in eukaryotes?
(A) Organization into a single operon transcribed from one promoter
(B) The presence of common specific cis-regulatory elements (e.g., response elements) in their promoters
(C) Transcription by a single massive RNA polymerase molecule
(D) Exclusive reliance on a single specific miRNA

2024

**188.** Which characteristic differentiates a silencer from a standard corepressor?
(A) A silencer is a DNA sequence, whereas a corepressor is a protein.
(B) A silencer is an RNA transcript, while a corepressor is a DNA element.
(C) Silencers act only on RNA polymerase I.
(D) Corepressors directly bind to the TATA box.

2007

**189.** A researcher identifies a novel DNA-binding protein. Structural analysis reveals an alpha helix and a beta sheet held together by a single structurally critical heavy metal ion. Which motif is this?
(A) Zinc finger
(B) Helix-turn-helix
(C) Leucine zipper
(D) bHLH

NODIA

2003

**190.** Why is the genetic code described as "unambiguous"?
(A) Because multiple codons can specify the same amino acid.
(B) Because a single specific codon never specifies more than one amino acid.
(C) Because it is identical in every known organism without exception.
(D) Because the reading frame can randomly shift.

2001

**191.** Which specific type of mutation fundamentally alters the reading frame of a translated mRNA?
(A) A single base transition
(B) A single base transversion
(C) A silent mutation in the wobble position
(D) A single base pair insertion or deletion

2010

**192.** Which cellular component strictly enforces the accurate translation of the genetic code prior to the ribosome's involvement?
(A) Aminoacyl-tRNA synthetases
(B) The spliceosome
(C) RNA polymerase II
(D) Poly-A polymerase

2011

**193.** The antibiotic puromycin inhibits translation by acting as an analog of:
(A) The 5' mRNA cap
(B) A release factor
(C) An aminoacyl-tRNA, causing premature chain termination
(D) The Shine-Dalgarno sequence

bond donors, acceptors, and hydrophobic patches unique to each base pair combination.

2010

**213.** The structural organization of a prokaryotic operon ensures that:
(A) Each gene is transcribed from its own individual promoter.
(B) Multiple metabolically related genes are transcribed as a single polycistronic mRNA unit.
(C) Introns are efficiently removed from the polycistronic mRNA.
(D) Translation occurs exclusively in the nucleus.

2011

**214.** Which specific molecular interaction is disrupted by the inducer allolactose in the lac operon system?
(A) The binding of CAP to the CAP site
(B) The binding of the LacI repressor to the operator
(C) The binding of RNA polymerase to the promoter
(D) The interaction between the beta and beta-prime polymerase subunits

2015

**215.** What limits the transcription of the lac operon when both glucose and lactose are present in the environment?
(A) The Lac repressor remains tightly bound to the operator.
(B) Low levels of cAMP prevent CAP from effectively binding and assisting RNA polymerase.
(C) Glucose physically blocks the entrance of the RNA polymerase to the DNA.
(D) Lactose is immediately degraded by extracellular enzymes.

2021

**216.** Which mechanistic factor explains how Trp operon attenuation senses the cellular concentration of tryptophan?
(A) By directly measuring the free tryptophan concentration using the RNA polymerase
(B) By monitoring the rate of DNA replication
(C) By the speed at which the ribosome translates tandem Trp codons in the leader peptide sequence
(D) By the interaction of a specific miRNA with the leader mRNA

2008

**217.** A genetic mutation that converts a UAG stop codon to a UAC tyrosine codon is classified as a:
(A) Missense mutation
(B) Silent mutation
(C) Nonsense mutation
(D) Frameshift mutation

2003

**218.** The redundancy of the genetic code is primarily accommodated by which property of translation?
(A) The specific wobble base pairing allowed at the third position of the codon
(B) The high error rate of peptidyl transferase
(C) The existence of 61 different release factors
(D) The skipping of nucleotides by the ribosome

2013

**219.** Which observation provided the earliest definitive evidence that DNA is a double-stranded, anti-parallel helix?
(A) X-ray crystallography data interpreted by Watson, Crick, and Franklin
(B) Cot curve analysis
(C) Recombinant DNA cloning
(D) The discovery of split genes

2004

**220.** Identify the INCORRECT statement regarding the kinetic classification of DNA reassociation.
(A) The fraction of DNA that renatures almost instantaneously consists of highly repetitive sequences.
(B) The moderately repetitive fraction includes functional multigene families like rRNAs.
(C) Unique DNA represents the fraction that reassociates at the highest Cot values.
(D) Genome size has absolutely no effect on the Cot curve profile.

2001

**221.** The phenomenon of "gene duplication followed by divergence" is a primary evolutionary source of:
(A) Novel alleles and multigene families
(B) Highly conserved housekeeping genes
(C) Universal RNA polymerase structures
(D) Identical redundant operons

2013

**222.** In identifying novel alleles in silico, which statistical tool evaluates the probability that a sequence alignment occurred by random chance?
(A) The $\mathrm{Cot}_{\frac{1}{2}}$ value
(B) The sedimentation coefficient
(C) The E-value (Expect value) in a BLAST

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defense mechanism will most likely target this viral intermediate?
(A) Processing by Dicer and targeting by the RNAi machinery
(B) Splicing via the spliceosome
(C) Polyadenylation by nuclear poly-A polymerase
(D) Direct translation by the 80S ribosome

2011

**260.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** The wobble hypothesis allows the genetic code to be translated efficiently by fewer than 61 tRNAs.
**Reason (R):** Non-Watson-Crick base pairing is permitted at the first position of the anticodon (corresponding to the third position of the codon).
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2011

**261.** In the sequence alignment of two evolutionary divergent paralogs, the regions that show the highest sequence conservation typically represent:
(A) Non-functional intronic regions
(B) The structural or catalytic domains essential for protein function
(C) Random synonymous mutations
(D) Telomeric repetitive DNA

2021

**262.** Which feature represents the most significant disadvantage of purely homology-based in silico gene discovery?
(A) It fails to identify genes with no known counterparts in existing databases (orphan genes).
(B) It strictly requires live organismal cultures.
(C) It is exceptionally slow and requires massive physical laboratory space.
(D) It can only identify highly mutated pseudogenes.

2005

**263.** When identifying cis-regulatory elements using bioinformatic approaches, researchers often look for:
(A) Long open reading frames
(B) Perfectly repetitive microsatellites
(C) Conserved, short non-coding sequence blocks upstream of homologous genes across species (phylogenetic footprinting)
(D) The presence of a poly-A tail in the genomic DNA

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2005

**264.** What fundamental limitation prevents a pure in silico gene model from being entirely definitive?
(A) In silico predictions cannot definitively confirm actual expression, post-transcriptional processing, or physiological functionality.
(B) Computer algorithms cannot process DNA sequences longer than 1000 bp.
(C) In silico databases exclusively contain RNA sequences.
(D) Eukaryotes do not use a standard genetic code.

2001

**265.** Which term describes the specialized DNA sequences at the physical ends of linear eukaryotic chromosomes that protect them from degradation?
(A) Centromeres
(B) Telomeres
(C) Insulators
(D) Origins of replication

2009

**266.** Which class of repetitive DNA constitutes the primary repetitive component of telomeres in most eukaryotes?
(A) SINEs
(B) LINEs
(C) Short tandem repeat satellite sequences
(D) Complex retroviral genomes

2025

**267.** What is the fundamental cause of the "end-replication problem" in linear eukaryotic genomes?
(A) RNA polymerase is not processive enough.
(B) DNA polymerase requires a primer and synthesizes exclusively 5' to 3', leaving a gap at the 5' end of the newly synthesized strand when the terminal RNA primer is removed.
(C) The presence of extreme GC-rich regions physically blocks the replication fork.
(D) Topoisomerases fail to relieve supercoiling at the ends.

polymerase

2023

**232.** Which characteristic definitively proves that two genes in a bacteriophage are "overlapping"?
(A) A specific nucleotide mutation simultaneously alters the amino acid sequence of two distinct viral proteins.
(B) They are transcribed from different strands of DNA.
(C) They share the exact same promoter sequence but are separated by a long intron.
(D) One gene is located entirely within the intron of another.

2009

**233.** What dictates the specific termination point of eukaryotic RNA polymerase II transcription?
(A) A strict hairpin loop similar to prokaryotic intrinsic termination
(B) It lacks a precise termination site and stops downstream of the poly-A cleavage signal, followed by exonuclease action (torpedo model).
(C) The binding of the rho protein
(D) It terminates instantly at the first UAA stop codon encountered in the DNA.

2008

**234.** In evaluating the "torpedo model" of transcription termination in eukaryotes, which enzyme plays the role of the "torpedo"?
(A) A 5'-to-3' exonuclease (like Xrn2) that degrades the downstream RNA attached to the polymerase
(B) A highly processive helicase that unwinds the DNA-RNA hybrid
(C) The poly-A polymerase itself
(D) The spliceosome

2014

**235.** Given below are four steps of a yeast two-hybrid assay:
I. Co-transform yeast with both plasmids.
II. Clone gene X in-frame with a DNA-binding domain (DBD).
III. Assess yeast growth on selective media lacking histidine.
IV. Clone gene Y in-frame with an activation domain (AD).
Which of the following represents the correct operational sequence?
(A) II, IV, I, III
(B) IV, II, III, I
(C) I, II, IV, III
(D) II, I, IV, III

2024

**236.** A significant advantage of homology-based gene cloning strategies is:
(A) They permit the rapid isolation of corresponding genes from unsequenced or non-model species using degenerate primers.
(B) They completely bypass the need for PCR.
(C) They physically assemble the proteins in vitro.
(D) They guarantee the discovery of genes with entirely novel functions unrelated to the probe.

NODIA

2017

**237.** Which eukaryotic transcription factor is structurally notable for containing a zinc finger domain and commonly binding as a homodimer to palindrome sequences?
(A) Steroid hormone receptors
(B) The TATA-binding protein
(C) The bacterial Lac repressor
(D) Ribosomal RNA polymerase

2010

**238.** What is the fundamental catalytic mechanism of a ribozyme during RNA splicing?
(A) It catalyzes transesterification reactions utilizing precisely positioned hydroxyl groups and metal ions.
(B) It requires the input of ATP to physically cut the RNA backbone.
(C) It synthesizes a new phosphodiester bond from free nucleotides.
(D) It methylates the branch point adenine to force dissociation.

2023

**239.** An investigator seeks to identify a silencer element regulating a specific gene. Which functional assay provides the most direct evidence of silencer activity?
(A) Northern blot of a housekeeping gene
(B) A reporter gene assay showing decreased expression when the element is cloned upstream of an active promoter
(C) Simple DNA sequencing of the entire region
(D) Measurement of total cellular RNA concentration

2006

**240.** The interaction between snRNPs $U_1$ and $U_2$ during spliceosome assembly fundamentally ensures:
(A) The addition of the poly-A tail

search
(D) The topological linking number

2011

**223.** What specific bioinformatic challenge do Hidden Markov Models (HMMs) uniquely solve in gene mining?
(A) Calculating exact DNA melting temperatures
(B) Identifying complex consensus motifs and domain profiles characteristic of divergent protein families
(C) Measuring exact cellular mRNA concentrations
(D) Visualizing real-time transcription

2011

**224.** Which structural modification marks a protein for degradation by the 26S proteasome?
(A) Polyubiquitination (specifically linked through Lysine 48)
(B) Monophosphorylation
(C) Extensive N-linked glycosylation
(D) Complete deacetylation

2019

**225.** Given below are five statements regarding eukaryotic translation initiation:
I. eIF4E binds the 5' cap.
II. The pre-initiation complex scans the mRNA for the Kozak consensus sequence.
III. The initiator tRNA carries methionine.
IV. The large 60S subunit joins after the start codon is recognized.
V. GTP hydrolysis is required for subunit joining.
Which of the above statements are correct?
(A) I, II, III and V
(B) I, II, IV and V
(C) II, III, IV and V
(D) I, III, IV and V

2009

**226.** How do upstream open reading frames (uORFs) typically regulate the translation of the main downstream reading frame?
(A) By recruiting massive numbers of ribosomes to enhance total mRNA activity
(B) By functioning as strong internal ribosome entry sites (IRES)
(C) By directly splicing the mRNA in the cytoplasm
(D) By causing the scanning ribosome to translate a short peptide and frequently dissociate before reaching the main start codon

2002

**227.** Which condition most potently activates the kinase that phosphorylates $eIF_2$, thereby shutting down global translation?
(A) Cellular stress such as amino acid starvation, viral infection, or ER stress
(B) High levels of intracellular ATP and nutrients
(C) Entry into the S phase of the cell cycle
(D) Binding of RNA polymerase II to a highly active promoter

2001

**228.** Which functional consequence is associated with the 2'-O-methylation of the 5' cap structure in higher eukaryotes?
(A) It marks the mRNA for immediate degradation.
(B) It helps the innate immune system distinguish self mRNA from viral RNA.
(C) It prevents the mRNA from leaving the nucleus.
(D) It initiates the splicing of the first intron.

2019

**229.** A researcher attempts to study protein-DNA interactions using EMSA. They run a native gel but fail to see a "shifted" band, although they confirm the protein binds the DNA tightly. What is the most likely technical reason for this failure?
(A) They used a radiolabeled DNA probe.
(B) They inadvertently added a strong denaturant (like SDS) to the gel.
(C) They used polyacrylamide instead of agarose.
(D) They incubated the sample at room temperature prior to loading.

2011

**230.** During ChIP analysis, an antibody against $H_3K_4me_3$ is used to pull down chromatin. Which genomic regions will primarily be enriched in this sample?
(A) Highly condensed telomeres
(B) Silenced centromeric DNA
(C) Active gene promoters
(D) Long unexpressed intergenic regions

2006

**231.** The concept of the "recon," proposed by Benzer, implies that recombination can occur:
(A) Only between two separate whole genes
(B) Between adjacent nucleotide pairs within a single gene
(C) Only between homologous chromosomes during meiosis
(D) Only in the presence of functional RNA

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List I: (Core Promoter Element)
P. TATA Box
Q. Initiator (Inr)
R. BRE
S. DPE
List II: (Functional Description)
1. Binds TFIIB, usually upstream of TATA
2. Binds TBP, highly AT-rich
3. Located downstream of the start site, often acts in TATA-less promoters
4. Encompasses the start site itself
(A) P-1, Q-2, R-3, S-4
(B) P-2, Q-4, R-1, S-3
(C) P-3, Q-1, R-4, S-2
(D) P-4, Q-3, R-2, S-1

2005
**251.** Which post-translational modification is primarily known for anchoring specific proteins to the cytosolic face of the cell membrane?
(A) Addition of a lipid anchor (e.g., myristoylation or farnesylation)
(B) Polyubiquitination
(C) N-linked glycosylation
(D) Acetylation

2024
**252.** The fundamental goal of "gene mining" in applied agricultural biotechnology is usually:
(A) Identifying naturally occurring alleles that confer resistance to stress or disease for crop improvement
(B) Eradicating all repetitive DNA from a crop
(C) Synthesizing entirely artificial genomes from scratch
(D) Studying the origin of viral overlapping genes

2012
**253.** Which sequence motif typically directs a newly translated protein to be imported into the nucleus?
(A) A short internal sequence rich in basic amino acids (lysine and arginine) known as an NLS
(B) A long hydrophobic signal peptide at the N-terminus
(C) A specific C-terminal KDEL sequence
(D) A poly-A sequence

2013
**254.** How does feedback inhibition in a biosynthetic pathway work?
(A) The final end product acts as an allosteric inhibitor of an early enzyme in the pathway.
(B) The final end product directly inhibits the transcription of the first enzyme's gene.
(C) The initial substrate binds to and destroys the final enzyme.
(D) The operon is deleted from the genome.

2003
**255.** How does alternative splicing bypass the constraints of genome size?
(A) By increasing the functional complexity of the proteome without requiring additional genes
(B) By radically increasing the rate of transcription
(C) By directly fusing multiple chromosomes together
(D) By allowing genes to be translated continuously without stop codons

2005
**256.** Which experimental outcome most strongly supports the existence of an insulator element?
(A) A sharp boundary of histone modifications is observed, preventing heterochromatin from spreading into an active gene.
(B) An enhancer increases transcription from a promoter located 10 kb away.
(C) A mutation in a repressor causes constitutive gene expression.
(D) The addition of lactose induces the lac operon.

2018
**257.** An enzyme specifically removes an acetyl group from the lysine residues of an $H_3$ histone tail. This enzyme is classified as a:
(A) Histone acetyltransferase (HAT)
(B) Histone deacetylase (HDAC)
(C) DNA methyltransferase (DNMT)
(D) ATP-dependent chromatin remodeler

2013
**258.** What is the most common phenotypic outcome of recruiting an HDAC complex to a promoter region?
(A) Transcriptional silencing and chromatin condensation
(B) Hyperactivation of transcription
(C) Rapid initiation of DNA replication
(D) Immediate splicing of all associated RNA transcripts

NODIA

2005
**259.** A novel RNA virus replicates in the cytoplasm and produces a highly structured double-stranded RNA during its life cycle. What host

(B) The spatial proximity of the 5' splice site and the branch point adenine for the first catalytic step
(C) The immediate export of the mRNA to the cytoplasm
(D) The absolute block of alternative splicing

2008

**241.** Which property best describes the nature of the genetic code's "punctuation"?
(A) The code is read continuously as triplets without any internal commas or spaces.
(B) The code utilizes specific non-coding nucleotides to separate adjacent codons.
(C) Introns act as punctuation marks between every single codon.
(D) Ribosomes pause for several seconds between each codon read.

2005

**242.** In determining the sequence of a novel polypeptide, the universal start codon AUG strictly establishes which parameter?
(A) The specific open reading frame to be translated
(B) The rate of transcription
(C) The exact length of the final poly-A tail
(D) The structural stability of the small ribosomal subunit

2006

**243.** The processivity of prokaryotic RNA polymerase is defined as:
(A) Its ability to continuously synthesize RNA without dissociating from the DNA template
(B) Its speed in finding the correct promoter
(C) The frequency at which it makes a transcription error
(D) Its absolute requirement for the sigma factor during elongation

2004

**244.** If an mRNA sequence reads 5'-AUCGUA-3', what was the sequence of the DNA template strand?
(A) 5'-AUCGUA-3'
(B) 5'-TAGCAT-3'
(C) 5'-TACGAT-3'
(D) 3'-TAGCAT-5'

2018

**245.** Which of the following defines a "trans-acting" regulatory element?
(A) A diffusible regulatory protein that can act on target genes located anywhere in the genome
(B) A specific DNA sequence in a promoter
(C) An enhancer located on the same chromosome
(D) An insulator element separating two chromatin domains

2011

**246.** Which structural feature most directly restricts a cis-acting element to regulating only closely linked genes?
(A) It requires a continuous DNA backbone to exert topological or physical influence on a nearby promoter.
(B) It acts via a diffusible RNA intermediate.
(C) It requires covalent modification by a kinase.
(D) It is an autonomous replicating sequence.

2004

**247.** The primary reason a missense mutation may not entirely abolish a protein's function is:
(A) Ribosomes can bypass mutated codons completely.
(B) The substituted amino acid may have similar biochemical properties (conservative substitution).
(C) The mutated mRNA is always degraded instantly.
(D) RNA polymerase corrects the mutation during a second round of transcription.

2006

**248.** In the regulation of the Trp operon, what specifically happens to the aporepressor when tryptophan is absent?
(A) It assumes an inactive conformation and cannot bind the operator DNA.
(B) It remains tightly bound to the operator.
(C) It acts as a powerful transcriptional activator.
(D) It initiates the attenuation mechanism.

2022

**249.** Which characteristic most effectively distinguishes an RNA polymerase from a typical DNA polymerase?
(A) RNA polymerase can initiate de novo strand synthesis without a primer.
(B) RNA polymerase requires a primer to initiate synthesis.
(C) RNA polymerase possesses highly efficient 3'-to-5' proofreading exonuclease activity.
(D) RNA polymerase uses strictly deoxyribonucleotides.

2015

**250.** Match the core RNA polymerase II promoter element in List I to its functional description in List II.

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2010
**268.** Telomerase solves the end-replication problem by acting biochemically as:
(A) A reverse transcriptase carrying its own RNA template
(B) A specialized DNA-dependent RNA polymerase
(C) A specific endonuclease
(D) An ATP-dependent DNA ligase

2001
**269.** The functional activity of the general transcription factor TFIIH is crucial for:
(A) Unwinding the DNA at the promoter (helicase) and phosphorylating the RNA polymerase II CTD (kinase)
(B) Adding the poly-A tail
(C) Transporting the mature mRNA out of the nucleus
(D) Catalyzing peptide bond formation

2001
**270.** A dominant negative mutation in a transcription factor typically operates by:
(A) Producing a non-functional mutant protein that blocks the activity of the normal wild-type protein, often by forming an inactive dimer
(B) Completely deleting the gene from the genome
(C) Causing the transcription factor to constantly activate every promoter
(D) Inducing immediate cell lysis

2005
**271.** In examining the regulatory network of a bacterial cell, which scenario best describes a global regulatory mechanism rather than a specific operon control?
(A) Regulation of the lac operon by lactose
(B) The stringent response mediated by (p) ppGpp, downregulating all rRNA synthesis during amino acid starvation
(C) The attenuation of the Trp operon
(D) The specific binding of the AraC protein

2022
**272.** A key physiological function of nonsense-mediated decay (NMD) in eukaryotes is to:
(A) Enhance the translation of short peptides
(B) Prevent the accumulation of potentially toxic truncated proteins resulting from premature stop codons
(C) Target perfectly normal mRNAs for regular cellular turnover
(D) Ensure that introns are efficiently translated

2022
**273.** How does the spliceosome distinguish a true splice site from numerous cryptic pseudo-splice sites present in a vast intron?
(A) By detecting the absence of a TATA box
(B) By the cooperative binding of SR proteins to exonic splicing enhancers (ESEs) that recruit spliceosomal components to the authentic boundaries
(C) By randomly cutting the RNA until the reading frame is correct
(D) By strictly measuring a fixed distance of exactly 100 nucleotides from the cap

2015
**274.** Which of the following accurately describes the structure of an RNA lariat intermediate?
(A) It features a unique 2'-5' phosphodiester bond connecting the 5' end of the intron to the branch point adenosine.
(B) It contains a strictly 5'-5' triphosphate linkage.
(C) It is entirely double-stranded RNA.
(D) It contains a perfectly linear continuous sequence of exons.

2021
**275.** An operon system engineered to lack a promoter sequence will result in:
(A) Constitutive high-level expression of all genes
(B) An inability of RNA polymerase to bind and initiate transcription, resulting in zero expression
(C) Transcription of only the first gene in the operon
(D) The ribosomes physically binding directly to the DNA to synthesize proteins

NODIA

2008
**276.** The wobble base pairing between codon UCU and an appropriate anticodon involves which position in the anticodon?
(A) The 5' most base of the anticodon
(B) The 3' most base of the anticodon
(C) The middle base of the anticodon
(D) The terminal CCA sequence

2012
**277.** The presence of a poly-A tail on a eukaryotic mRNA most significantly affects:
(A) The halF-life of the transcript and its efficiency of translation
(B) The physical location of transcription initiation
(C) The rate of RNA splicing

(B) They occur simultaneously in the cytoplasm.
(C) Translation strictly precedes transcription.
(D) Both processes are carried out by the exact same multi-enzyme complex.

2008

**297.** The phenomenon of transcriptional attenuation relies entirely on which physical parameter?
(A) The tight spatial and temporal coupling of RNA polymerase advancing on DNA and the ribosome advancing on the nascent RNA transcript
(B) The continuous rapid export of mRNA from the nucleus
(C) The rapid degradation of the sigma factor
(D) The specific binding of the CAP protein

2013

**298.** Which specific enzymatic process characterizes the "capping" of eukaryotic pre-mRNA?
(A) A guanylyltransferase adding a GMP in a 5'-5' linkage followed by methylation
(B) A kinase phosphorylating the 3' end
(C) An exonuclease trimming the 5' end
(D) A ligase joining two separate primary transcripts

2025

**299.** In evaluating post-transcriptional gene regulation, the binding of the RISC complex to a target mRNA most frequently leads to:
(A) Transcript cleavage or the severe repression of successful translation
(B) The massive stabilization and prolonged halF-life of the mRNA
(C) Enhanced rapid export from the nucleus
(D) Spontaneous splicing of remaining introns

2021

**300.** The application of Hidden Markov Models (HMM) in modern gene mining algorithms is highly favored due to their ability to:
(A) Statistically score the probability of a sequence belonging to a specific conserved protein family using complex position-specific profiles
(B) Guarantee 100% accurate structural folding of any protein sequence
(C) Instantly synthesize the corresponding DNA sequence in the laboratory
(D) Visualize the entire chromatin structure of a live cell at atomic resolution

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(B) Nonsense-mediated decay
(C) Immediate secretion into the extracellular matrix
(D) Conversion into a stable prion

2014
**288.** A researcher identifies a short RNA of ~22 nucleotides that exhibits imperfect complementarity to the 3' UTR of multiple genes. This molecule is almost certainly:
(A) A microRNA (miRNA)
(B) A small interfering RNA (siRNA)
(C) A small nuclear RNA (snRNA)
(D) A transfer RNA (tRNA)

2013
**289.** Which defining characteristic best separates classical Mendelian concepts of the gene from modern molecular definitions involving split genes?
(A) Classical genes were assumed to be continuous linear units of information, while modern molecular genes may be discontinuous sequences separated by introns.
(B) Classical genes were believed to be made of RNA, while modern ones are DNA.
(C) Classical genes did not dictate phenotypes.
(D) Classical definitions included precise promoter locations.

2013
**290.** What specific mechanistic role does the 16S rRNA perform in assuring translational fidelity?
(A) It monitors the geometry of the codon-anticodon pairing in the minor groove of the A site.
(B) It catalyzes the formation of the peptide bond.
(C) It chemically modifies incorrect amino acids.
(D) It actively cuts the mRNA if a mutation is detected.

2007
**291.** Why does alternative splicing play a significantly larger role in the phenotypic complexity of higher eukaryotes compared to simple eukaryotes like yeast?
(A) Higher eukaryotes possess substantially more introns per gene and complex regulatory splicing factors allowing diverse isoform generation.
(B) Yeast completely lacks a spliceosome.
(C) Higher eukaryotes replicate their DNA much faster.
(D) Yeast genes are not transcribed into mRNA.

2012
**292.** The addition of ubiquitin molecules to a target protein is a sequential cascade catalyzed by three enzymes: $E_1$, $E_2$, and $E_3$. Which of these specifically dictates substrate recognition and specificity?
(A) $E_3$ (Ubiquitin ligase)
(B) $E_1$ (Ubiquitin-activating enzyme)
(C) $E_2$ (Ubiquitin-conjugating enzyme)
(D) The 26S proteasome itself

2023
**293.** Which technique provides the most robust bioinformatic validation of an computationally predicted gene model structure?
(A) Aligning full-length cDNA or RNA-seq data against the genomic sequence
(B) Measuring the precise length of the entire chromosome
(C) Determining the GC content of the genome
(D) Running an EMSA on the promoter region

2005
**294.** A "motif" in the context of transcription factors is strictly defined as:
(A) A distinct, recurrent 3D structural folding pattern that typically mediates specific DNA binding or dimerization
(B) The entire length of the polypeptide chain
(C) The random coil regions of the protein
(D) The specific mRNA sequence that translated the protein

NODIA

2007
**295.** Which primary difference distinguishes intrinsic from rho-dependent termination structurally?
(A) Intrinsic termination relies on the specific formation of an RNA hairpin followed by a weak poly-U tract, while rho-dependent lacks the poly-U requirement and requires a rut site.
(B) Intrinsic termination relies on a specific sequence in the DNA template, while rho-dependent happens entirely randomly.
(C) Intrinsic termination involves the spliceosome.
(D) Rho-dependent termination only occurs in eukaryotic cells.

2005
**296.** In a typical eukaryotic cell, what defines the spatial and temporal relationship between transcription and translation?
(A) Transcription occurs in the nucleus, followed by mRNA export, and subsequent translation in the cytoplasm.

(D) The specific amino acid sequence of the encoded protein

2007

**278.** Which statement about the universal genetic code is functionally correct?
(A) It provides a near-universal dictionary translating 64 nucleotide triplets into 20 amino acids and 3 stop signals.
(B) It lacks redundancy completely.
(C) A specific codon can specify multiple distinct amino acids in the same cell.
(D) It overlaps continuously in all eukaryotic structural genes.

2025

**279.** The fundamental purpose of analyzing the $Cot_{\frac{1}{2}}$ value of a DNA sample is to:
(A) Determine the exact length of the chromosome
(B) Assess the kinetic complexity, reflecting the total length of unique sequences in the genome
(C) Measure the efficiency of translation
(D) Determine the specific number of introns in a gene

2023

**280.** A repressor binding to an operator strictly exemplifies which specific type of regulatory control mechanism?
(A) Negative transcriptional control
(B) Post-translational modification
(C) Transcriptional attenuation
(D) RNA interference

2013

**281.** The formation of a eukaryotic translation initiation complex relies on a loop structure where the 5' cap interacts with the 3' poly-A tail. Which protein mediates this bridging interaction?
(A) eIF4G
(B) $eIF_2$
(C) Peptidyl transferase
(D) $eRF_1$

2018

**282.** During severe cellular stress, phosphorylation of $eIF_2$ alpha prevents translation initiation by:
(A) Destroying the 40S ribosomal subunit
(B) Acting as a competitive inhibitor of the $eIF_2B$ guanine nucleotide exchange factor, trapping $eIF_2$ in an inactive GDP-bound state
(C) Directly cleaving the 5' cap off all mRNAs
(D) Immediately initiating the apoptotic cascade

2024

**283.** In identifying an unknown DNA sequence as a potential enhancer, the defining characteristic required is that it:
(A) Can stimulate transcription from a basal promoter irrespective of its orientation or considerable distance
(B) Absolutely requires a TATA box within itself
(C) Must bind the RNA polymerase core enzyme directly
(D) Must be transcribed into a highly stable small nuclear RNA

2019

**284.** Which statement represents the best interpretation of a multiphasic Cot curve?
(A) The sample contains only a single type of viral genome.
(B) The DNA contains distinct classes of sequences differing widely in their repetition frequencies.
(C) The DNA is highly degraded and cannot renature.
(D) The genome lacks any structural genes.

2012

**285.** A novel protein is discovered that specifically recognizes heavily methylated CpG dinucleotides. This protein most likely functions to:
(A) Initiate DNA replication
(B) Recruit chromatin-remodeling complexes that establish a repressive heterochromatin state
(C) Serve as a highly active basal transcription factor
(D) Actively repair UV-induced thymine dimers

2006

**286.** What functional capability characterizes the action of a "ribozyme"?
(A) Acting as an RNA molecule with intrinsic catalytic enzymatic activity
(B) Transporting amino acids to the ribosome
(C) Carrying genetic information from the nucleus to the cytoplasm
(D) Acting as a structural scaffold for the plasma membrane

2004

**287.** Which process ensures that an improperly folded protein in the endoplasmic reticulum does not accumulate and cause toxicity?
(A) ER-associated degradation (ERAD) involving retrotranslocation and ubiquitination

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# ANSWERS

**1.** (A) Kinetic complexity of the genome
The parameter describing the point where half the DNA in a sample has reassociated is known as the halF-Cot value. This value is directly proportional to the kinetic complexity of the genome, representing the total length of unique sequences. It allows researchers to estimate genome size and identify the proportion of repetitive DNA within different organisms.

**2.** (C) Concentration of unique sequences
In complex eukaryotic genomes, the rate of DNA renaturation is primarily determined by the concentration of unique sequences. Since unique sequences are present in fewer copies, they take longer to find their complementary strands compared to repetitive sequences. This principle underlies the mathematical analysis of reassociation kinetics, providing insights into the structural organization and repetition frequency of DNA.

**3.** (A) HalF-Cot value
The halF-Cot value is the most appropriate indicator of genome complexity derived from DNA reassociation kinetics. It represents the product of the initial DNA concentration and the time required for half of the sample to renature. Larger genomes with more unique sequences exhibit higher values, reflecting their greater kinetic complexity compared to smaller or highly repetitive genomic structures.

**4.** (D) The fast-reassociating fraction represents unique sequences.
Eukaryotic Cot curves typically reveal multiple phases representing different repetition frequencies. While repetitive DNA reassociates rapidly at low values, unique sequences require much higher values due to their lower concentration. The fast-reassociating fraction represents highly repetitive sequences rather than unique ones. Genome size significantly influences the renaturation kinetics of the unique fraction, which follows a predictable second-order reaction.

**5.** (A) Both A and R are true and R is the correct explanation of A.
Eukaryotic genomes exhibit multiphasic Cot curves because they contain sequences with varying levels of repetition, including unique, moderately repetitive, and highly repetitive DNA. Each fraction reassociates at a distinct rate based on its concentration in the total DNA sample. This diversity in repetition frequency creates the complex, stepped profile characteristic of eukaryotic renaturation kinetics, reflecting their overall structural organization.

**6.** (C) Satellite DNA
Satellite DNA consists of highly repetitive, short tandem sequences that reassociate almost instantaneously during renaturation experiments. Because these sequences are present in enormous copy numbers, they encounter complementary strands very frequently. Consequently, this fraction is characterized by the lowest Cot values in a standard kinetics curve, distinguishing it from moderately repetitive sequences and the much slower-reassociating unique genomic regions.

**7.** (D) Inversely proportional
The renaturation rate of DNA is inversely proportional to the sequence length of the repeating unit. As the complexity or length of the unique sequence increases, the probability of complementary strands finding each other decreases. This mathematical relationship is fundamental to understanding why smaller, simpler viral genomes renature much faster than the vastly more complex and longer genomes of eukaryotes.

**8.** (A) Presence of LINEs and SINEs
The intermediate reassociating fraction in a eukaryotic Cot curve corresponds to moderately repetitive DNA. This class includes interspersed sequences such as Long Interspersed Nuclear Elements and Short Interspersed Nuclear Elements. These sequences reassociate faster than unique DNA but slower than highly repetitive satellite DNA. Analysis of this fraction provides essential information regarding

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**44.** (D) Rho-dependent termination fails to occur.
The rut site serves as the primary binding location for the rho protein on the nascent RNA transcript. If a mutation deletes this site, the rho protein cannot attach to the RNA and move toward the transcription complex. Consequently, rho-dependent termination fails to occur, and the RNA polymerase may continue transcribing past the normal termination point into downstream genomic regions.

**45.** (A) Both A and R are true and R is the correct explanation of A.
Eukaryotic RNA polymerase II is highly sensitive to alpha-amanitin, a toxin from poisonous mushrooms. The toxin specifically binds to the enzyme and inhibits the translocation step, thereby preventing RNA elongation. This biochemical sensitivity is a defining characteristic used to distinguish polymerase II from other eukaryotic RNA polymerases, which show either moderate sensitivity or complete resistance to the effects of the toxin.

**46.** (C) 28S, 18S, and 5.8S ribosomal RNA (rRNA)
Eukaryotic RNA polymerase I is specialized for the synthesis of the large ribosomal RNA precursor. This transcript is subsequently processed into the 28S, 18S, and 5.8S rRNA subunits, which are essential components of the ribosome. Because rRNA constitutes the majority of cellular RNA, RNA polymerase I activity is localized within the nucleolus and accounts for a significant portion of transcription.

**47.** (D) It requires the TATA-binding protein exclusively for termination.
RNA polymerase III synthesizes small RNAs, including 5S rRNA and tRNAs, often using internal promoters located downstream of the start site. While it exhibits varying sensitivity to alpha-amanitin, it does not require the TATA-binding protein exclusively for termination. TBP is actually part of the initiation complex for all eukaryotic polymerases, while termination involves specific internal signals within the RNA transcript.

**48.** (C) They contain 10-14 subunits with species-specific components.
Eukaryotic RNA polymerases are significantly more complex than the prokaryotic core enzyme. They are composed of ten to fourteen different subunits, including several that are shared among all three types and others that are species-specific. This multi-subunit structure allows for sophisticated regulation and coordination with various transcription factors, reflecting the greater complexity of gene expression control in eukaryotic organisms.

**49.** (A) RNA Polymerase I
The sensitivity of eukaryotic RNA polymerases to alpha-amanitin varies significantly between the different types. RNA polymerase I, which is responsible for synthesizing ribosomal RNA in the nucleolus, is completely insensitive to this toxin, even at high concentrations. In contrast, RNA polymerase II is highly sensitive, and RNA polymerase III shows moderate sensitivity, allowing researchers to selectively inhibit specific classes of transcription.

**50.** (A) $-25$ to $-30$
In the eukaryotic core promoter, the TATA box is a highly conserved AT-rich sequence. It is typically located at a relative position of $-25$ to $-30$ base pairs upstream of the transcription start site. This element is recognized by the TATA-binding protein, which initiates the assembly of the pre-initiation complex, ensuring the precise positioning of RNA polymerase II for initiation.

**51.** (B) TFIID
TFIID is a basal transcription factor that plays a central role in eukaryotic transcription initiation. It is a large multi-subunit complex that contains the TATA-binding protein and various TBP-associated factors. TFIID is the first factor to bind the promoter, recognizing the TATA box or other core elements, and serves as the foundation for the subsequent assembly of the pre-initiation complex.

**52.** (C) TATA-less promoters
The Downstream Promoter Element is a core promoter motif typically found in TATA-less promoters of eukaryotic genes. It is located approximately thirty nucleotides downstream of the transcription start site. The DPE works in conjunction with the Initiator element to provide a binding site for TFIID, allowing for the accurate initiation of transcription in genes that lack a traditional TATA box.

NODIA

**17.** (A) It is highly transcribed into mRNA.
Highly repetitive DNA is typically found in heterochromatic regions and is generally transcriptionally silent, meaning it is not highly transcribed into mRNA. It often consists of short tandem repeats and forms distinct satellite bands during density gradient centrifugation. While it constitutes a large fraction of many eukaryotic genomes, its functions are primarily structural, involving centromere stability rather than encoding cellular proteins.

**18.** (C) A structural gene encoding a polypeptide
The classical genetic term "cistron" refers to a unit of function defined by the cis-trans complementation test. In modern molecular biology, it is functionally equivalent to a structural gene that encodes a single polypeptide. While the discovery of introns and alternative splicing has refined our understanding, the cistron remains a fundamental concept for describing the genetic segment responsible for protein synthesis.

**19.** (A) The smallest sequence of DNA capable of mutation
Seymour Benzer introduced the term "muton" to define the smallest unit of DNA capable of mutation. Molecularly, this corresponds to a single nucleotide pair. His work with bacteriophages demonstrated that mutations are not indivisible units but occur at the level of individual bases. This discovery was critical for transitioning from classical genetics to a physical understanding of the DNA double helix.

**20.** (D) Recon
The term "recon" defines the smallest unit of DNA that can undergo genetic recombination. Like the muton, the recon can be as small as a single nucleotide pair. This concept illustrates that recombination can occur at any point along the DNA sequence, even within the boundaries of a single gene or cistron, allowing for high levels of genetic diversity and variation.

**21.** (B) It showed that a single continuous sequence is not required to encode a protein.
The discovery of split genes revealed that eukaryotic coding sequences are interrupted by non-coding introns. This modified the classical definition of a cistron by demonstrating that a single continuous DNA sequence is not required to encode a protein. Instead, exons must be spliced together after transcription, showing that genetic information is often stored in a mosaic rather than a contiguous format.

**22.** (C) Increased protein diversity through alternative splicing
The split gene architecture provides a significant evolutionary advantage by enabling alternative splicing. This process allows a single gene to produce multiple distinct mRNA transcripts and protein isoforms by selectively including or excluding various exons. Consequently, organisms can greatly increase their protein diversity and functional complexity without a corresponding increase in the total number of genes within their genome, enhancing adaptability.

**23.** (A) Variations in non-coding and repetitive DNA content
The $C$-value paradox refers to the lack of correlation between the total DNA content of a genome and the biological complexity of the organism. This discrepancy is primarily caused by variations in non-coding and repetitive DNA content. While the number of essential genes remains relatively similar across many taxa, the amount of satellite DNA and transposable elements varies dramatically between species.

**24.** (B) Promoter and terminator
A basic transcription unit is defined by structural components that dictate where RNA synthesis starts and ends. The promoter serves as the specific binding site for RNA polymerase, marking the beginning of the unit. The terminator sequence signals the enzyme to cease elongation and release the nascent transcript. These two elements are absolutely essential for maintaining the integrity and boundaries of gene expression.

**25.** (B) II and III
In a typical eukaryotic transcription unit, the promoter is located upstream of the transcription start site, and terminators dictate the end of RNA synthesis. However, the coding sequence is rarely continuous because it is interrupted by introns. Therefore, statements regarding the promoter and terminator are correct, while the claim of a continuous coding sequence is inaccurate for most eukaryotic structural genes.

**26.** (A) Frequent initiation of transcription
A strong promoter is characterized by a high affinity for RNA polymerase and its associated transcription factors. This leads to the frequent

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the distribution and evolutionary history of transposable elements.

**9.** (B) Pseudogenes and paralogs
The determination of the true unique gene number is complicated by the presence of pseudogenes and paralogs. These sequences share high similarity with functional genes but often result from duplication events or accumulation of mutations. Because they can reassociate with the unique fraction or represent moderate repetition, they make it difficult to distinguish between functional coding sequences and non-functional genetic remnants.

**10.** (B) Polyploidy and genome duplication
Polyploidy and genome duplication events complicate the interpretation of genome complexity based solely on Cot curves. These phenomena increase the total DNA content without necessarily adding new unique information. While the total $C$-value increases, the kinetic complexity may remain similar to a diploid ancestor, leading to potential discrepancies when correlating DNA mass with the functional number of unique gene sequences.

**11.** (A) Highly repetitive satellite DNA
Centromeric heterochromatin is predominantly composed of highly repetitive satellite DNA sequences arranged in tandem. These regions are characterized by a lack of structural genes and a highly condensed chromatin state. The repetitive nature of this DNA allows for rapid reassociation in kinetics studies. Its primary function involves providing structural stability to the chromosome and facilitating proper spindle attachment during cell division.

**12.** (C) SINEs
Alu elements are the most abundant class of Short Interspersed Nuclear Elements found in the human genome. These non-autonomous retrotransposons are approximately three hundred base pairs long and rely on the enzymatic machinery of other elements for mobility. They are categorized as moderately repetitive DNA and are widely dispersed across chromosomes, playing significant roles in genomic evolution and occasionally causing genetic disorders.

**13.** (D) LINEs encode proteins for their own retrotransposition.
Long Interspersed Nuclear Elements are distinguished from Short Interspersed Nuclear Elements by their autonomy. LINEs encode the necessary proteins, such as reverse transcriptase and endonuclease, required for their own retrotransposition. In contrast, SINEs lack these coding sequences and must hijack the enzymatic machinery produced by LINEs to move within the genome. This functional independence is a defining characteristic of LINEs.

**14.** (C) RNA-mediated transposition
The expansion of moderately repetitive DNA sequences, particularly LINEs and SINEs, is primarily driven by RNA-mediated transposition. This process involves the transcription of the element into an RNA intermediate, which is then reverse-transcribed back into DNA and integrated at a new genomic location. This "copy and paste" mechanism allows these elements to increase their copy number significantly over evolutionary timescales.

NODIA

**15.** (B) P-3, Q-1, R-2, S-4
DNA sequences are classified based on their repetition and function. Short Interspersed Nuclear Elements are non-autonomous retrotransposons, while satellite DNA consists of tandem repeats in centromeres. Unique DNA contains structural genes encoding proteins, and Long Interspersed Nuclear Elements act as autonomous retrotransposons. Matching these features correctly clarifies the diverse structural organization found within eukaryotic genomes, distinguishing between coding and repetitive elements.

**16.** (D) Microsatellites – Dispersed throughout the genome
Microsatellites, also known as simple sequence repeats, consist of short motifs tandemly repeated and are dispersed throughout the genome. Unlike satellite DNA, which is concentrated in centromeres, microsatellites occur in both coding and non-coding regions. Their high polymorphism makes them invaluable markers for genetic mapping, DNA profiling, and studying population genetics, as they vary significantly in length between individuals and species.

most genes during normal growth. Different sigma factors are activated under specific stress conditions to redirect RNA polymerase to new sets of promoters. However, sigma factors do not remain attached during elongation and lack intrinsic helicase activity. Therefore, statements regarding their housekeeping role and stress activation are correct.

**36.** (C) Specific binding to promoter sequences
The critical functional distinction between the core enzyme and the holoenzyme of prokaryotic RNA polymerase is the ability to perform specific binding to promoter sequences. The core enzyme can synthesize RNA and elongate transcripts but lacks the specificity to identify promoters. The addition of the sigma factor to form the holoenzyme is necessary to direct the polymerase to initiate transcription accurately.

**37.** (A) Sigma 32
In Escherichia coli, Sigma 32 is the specialized sigma factor primarily responsible for transcribing heat shock genes. When the cell experiences elevated temperatures, the concentration and activity of Sigma 32 increase, allowing RNA polymerase to recognize promoters of genes encoding chaperones and proteases. This response helps the bacterium survive protein denaturation and maintain cellular homeostasis under stressful environmental conditions.

**38.** (D) Unwinding of DNA around the transcription start site
The transition from a closed promoter complex to an open promoter complex during prokaryotic transcription initiation involves the localized unwinding of the DNA double helix. This process occurs around the transcription start site, creating a transcription bubble that exposes the template strand. This melting of DNA is essential for RNA polymerase to begin the synthesis of the nascent RNA transcript using ribonucleotides.

**39.** (A) Release of short RNA transcripts before promoter escape
Abortive initiation is a characteristic phase of transcription where RNA polymerase releases short RNA transcripts, typically less than ten nucleotides long, before successfully escaping the promoter. During this stage, the enzyme remains engaged at the promoter while repeatedly attempting to initiate synthesis. Successful promoter escape marks the end of abortive cycles and the beginning of the processive elongation phase of transcription.

**40.** (B) The topological stability of the transcription bubble
High processivity during the elongation cycle of prokaryotic transcription is ensured by the topological stability of the transcription bubble. The RNA polymerase maintains a tight grip on both the DNA template and the nascent RNA transcript within a stable complex. This configuration prevents the premature dissociation of the enzyme, allowing it to synthesize long RNA molecules without interruption across the gene.

**41.** (A) A GC-rich hairpin followed by a poly-U sequence in the RNA
Intrinsic or rho-independent termination requires a specific structural feature in the emerging RNA transcript. This consists of a GC-rich hairpin structure followed by a sequence of uracil residues. The stable hairpin causes the RNA polymerase to pause, while the weak adenine-uracil base pairs between the template and the transcript facilitate the dissociation of the RNA from the DNA and the enzyme.

**42.** (B) It binds the rut site as an ATP-dependent RNA-DNA helicase.
The rho protein facilitates transcription termination by binding to a specific site on the nascent RNA known as the rut site. Acting as an ATP-dependent RNA-DNA helicase, it moves along the transcript toward the 3′ end. Once it reaches the stalled RNA polymerase, it uses its helicase activity to unwind the RNA-DNA hybrid, triggering the release of the transcript.

**43.** (A) The requirement for a trans-acting protein factor
The fundamental difference between rho-dependent and rho-independent termination is the requirement for a trans-acting protein factor. Rho-independent termination relies solely on the intrinsic secondary structure of the RNA transcript and the DNA template sequence. In contrast, rho-dependent termination necessitates the action of the rho protein, which actively tracks the RNA to facilitate the dissociation of the transcription complex from DNA.

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represents an incorrect pair in standard genetic mapping.

**81.** (D) 30S subunit
The 30S subunit of the prokaryotic ribosome is responsible for the decoding of the mRNA template. It contains the 16S rRNA and numerous proteins that form the interface for codon-anticodon interaction. This subunit ensures the accurate matching of incoming aminoacyl-tRNAs with the genetic instructions. Once the correct tRNA is selected, the large subunit facilitates the catalytic steps of protein synthesis.

**82.** (B) 60S and 40S
The typical eukaryotic ribosome is characterized by a sedimentation coefficient of 80S. It is composed of two major subunits: the large 60S subunit and the small 40S subunit. The 40S subunit handles mRNA decoding, while the 60S subunit contains the peptidyl transferase center. Together, they coordinate the complex process of translation, facilitating the assembly of amino acids into polypeptides.

**83.** (B) A cloverleaf
The secondary structure of transfer RNA is universally described as resembling a cloverleaf shape. This model is formed by internal base pairing that creates several distinct arms and loops, including the D loop, T-psi-C loop, and the anticodon loop. This folding is essential for its function as an adapter molecule, allowing it to interact specifically with aminoacyl-tRNA synthetases and ribosomes.

**84.** (B) The 3′ CCA acceptor stem
The 3′ CCA acceptor stem is the functional region of the tRNA molecule where the specific amino acid is covalently attached. This conserved sequence at the 3′ end provides the site for the esterification reaction catalyzed by aminoacyl-tRNA synthetases. The attachment of the amino acid is the first critical step in ensuring that the genetic code is translated with high fidelity.

**85.** (A) Both A and R are true and R is the correct explanation of A.
Aminoacyl-tRNA synthetases are highly specific enzymes that play a vital role in maintaining the fidelity of translation. They must accurately pair each specific amino acid with its corresponding tRNA molecule based on both the anticodon and structural features. This precise matching ensures that the correct amino acids are incorporated into the growing polypeptide chain according to the instructions in the mRNA.

**86.** (B) A proofreading editing site that hydrolyzes incorrectly paired amino acids
Some aminoacyl-tRNA synthetases possess a proofreading mechanism to correct mischarging errors. This involves a separate editing site that identifies and hydrolyzes amino acids that have been incorrectly paired with a tRNA. By checking the size and chemical properties of the amino acid before the tRNA is released, these enzymes significantly reduce the error rate of protein synthesis, ensuring high translational accuracy.

**87.** (A) The 16S rRNA of the 30S subunit
During prokaryotic translation initiation, the Shine-Dalgarno sequence in the mRNA base-pairs with a complementary region at the 3′ end of the 16S rRNA. This interaction is mediated by the 30S ribosomal subunit and ensures the precise positioning of the start codon in the P site. This mechanism allows the ribosome to identify the correct initiation point in polycistronic transcripts.

**88.** (C) eIF4E
eIF4E is the specific initiation factor that binds to the 5′ cap of eukaryotic mRNA during the earliest stages of translation. This binding is critical for the recruitment of the 43S pre-initiation complex to the transcript. By interacting with other factors like eIF4G, eIF4E facilitates the assembly of the machinery required for the ribosome to scan and locate the start.

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**89.** (B) The A (Aminoacyl) site
In the elongating ribosome, an incoming aminoacyl-tRNA first enters the A site. This site is responsible for monitoring the codon-anticodon interaction to ensure that the correct amino acid is delivered. Once the pairing is verified, the polypeptide chain is transferred to the new amino acid, and the ribosome translocates to move the tRNA from the A site to the P.

**90.** (B) Peptidyl transferase
Peptidyl transferase is the enzymatic activity directly responsible for the formation of peptide bonds during translation. It catalyzes the

**53.** (D) Helicase and kinase activity
TFIIH is a multi-functional basal transcription factor essential for the formation of the eukaryotic pre-initiation complex. It provides critical helicase activity to unwind the DNA at the promoter and kinase activity to phosphorylate the C-terminal domain of RNA polymerase II. These enzymatic functions are required for the transition from initiation to the elongation phase, allowing the polymerase to escape.

**54.** (D) The transcription start site
The Initiator element is a core promoter sequence that encompasses the transcription start site in eukaryotic genes. It is characterized by a loosely conserved consensus sequence that spans the +1 position. The Inr element is recognized by subunits of TFIID and can function independently or in cooperation with the TATA box to recruit the transcription machinery and define the initiation point.

**55.** (A) P-2, Q-3, R-4, S-1
Regulatory elements serve distinct roles in eukaryotic transcription. Enhancers increase the transcription rate independent of orientation, while silencers actively repress it. Insulators function as boundaries that block inappropriate interactions between enhancers and promoters. The TATA-binding protein is a basal factor that specifically binds the TATA box. Correctly matching these components highlights the complex interplay of factors that control spatial and temporal gene expression.

**56.** (B) They function in an orientation and distance-independent manner.
Eukaryotic enhancers are cis-regulatory elements that function in an orientation and distance-independent manner. They can be located thousands of base pairs away from the promoter, either upstream or downstream, and still significantly increase the rate of transcription. Enhancers work by binding specific activator proteins that interact with the basal transcription machinery through chromatin looping, facilitating the assembly of the initiation complex.

**57.** (A) By establishing physical boundaries in chromatin looping
Insulator elements typically function by establishing physical boundaries in chromatin looping. They bind specific proteins, such as CTCF in mammals, which organize the DNA into structural domains. This organization prevents an enhancer in one domain from interacting with a promoter in an adjacent domain. By regulating these spatial interactions, insulators ensure that genes are only activated by their appropriate regulatory elements.

**58.** (A) By recruiting corepressors that induce repressive chromatin marks.
Eukaryotic silencer elements exert their repressive effect on transcription by recruiting corepressor proteins. these corepressors often induce repressive chromatin marks, such as histone deacetylation or specific methylation patterns, which lead to chromatin condensation. This structural change makes the DNA less accessible to RNA polymerase and general transcription factors, thereby significantly reducing or completely inhibiting the initiation of RNA synthesis.

**59.** (C) Helix-turn-helix
The helix-turn-helix motif is a classic DNA-binding domain that typically utilizes an alpha helix inserted into the major groove of the DNA. This recognition helix forms specific hydrogen bonds with the bases, allowing the protein to identify its target sequence. The motif is characterized by two alpha helices joined by a short turn, providing a stable scaffold for DNA interaction.

**60.** (A) Cysteine and Histidine
The zinc finger motif is a common DNA-binding structure stabilized by the coordination of a central zinc ion. This ion is typically held in place by specific amino acid residues, most commonly pairs of cysteine and histidine. The coordination of the metal ion allows a small protein domain to fold into a stable, finger-like structure that fits into the DNA.

**61.** (B) Dimerization of alpha-helices facilitating DNA interaction.
The leucine zipper motif provides a structural basis for DNA binding through the dimerization of two alpha-helices. Each helix contains leucine residues at every seventh position, which interdigitate to form a hydrophobic “zipper” interface. This dimerization creates a Y-shaped structure where the basic N-terminal regions of both helices insert into the major groove of the DNA, facilitating sequence-specific interaction.

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**62.** (D) A basic region for DNA binding and an HLH domain for dimerization.
The basic helix-loop-helix transcription factor is characterized by two distinct functional domains. It contains a basic region at the N-terminus that mediates specific DNA binding in the major groove. This is followed by an HLH domain, consisting of two alpha helices separated by a loop, which facilitates dimerization. Dimerization is essential for the factor to bind DNA stably and regulate transcription.

**63.** (A) Addition of 7-methylguanosine via a $5' - 5'$ triphosphate link.
The $5'$ capping of eukaryotic mRNA involves the addition of a 7-methylguanosine residue to the first nucleotide of the nascent transcript. This occurs via a unique $5' - 5'$ triphosphate linkage, which is distinct from standard phosphodiester bonds. This modification is catalyzed by a series of enzymes that associate with the C-terminal domain of RNA polymerase II shortly after the initiation of transcription.

**64.** (A) It prevents $5'$ to $3'$ degradation and aids translation.
The $5'$ mRNA cap serves several critical biological functions in eukaryotes. Primarily, it protects the transcript from degradation by $5'$ to $3'$ exonucleases, thereby increasing its stability. Additionally, the cap is recognized by translation initiation factors, which facilitate the recruitment of the ribosome to the mRNA. It also plays an essential role in the efficient export of mature mRNA.

**65.** (C) I and III
The $3'$ polyadenylation of eukaryotic mRNA is a critical processing step. It requires the highly conserved consensus sequence AAUAAA, which signals for cleavage and the subsequent addition of the poly-A tail. This tail protects the transcript from rapid exonucleolytic degradation. However, poly-A polymerase does not require an RNA template, as it adds adenine nucleotides directly to the $3'$ end of the mRNA.

**66.** (B) 10-30 nucleotides downstream
In eukaryotes, the $3'$ cleavage of the pre-mRNA transcript generally occurs at a specific site ten to thirty nucleotides downstream of the AAUAAA consensus signal. This cleavage is performed by an endonuclease complex that recognizes the signal and an additional downstream GU-rich element. Once the transcript is cut, poly-A polymerase immediately begins adding a string of adenine residues to the new end.

**67.** (B) Extensive capping, splicing, and polyadenylation of transcripts.
The maturation of mRNA in eukaryotes is fundamentally distinguished from prokaryotes by extensive post-transcriptional processing. In eukaryotes, transcripts undergo $5'$ capping, splicing to remove introns, and $3'$ polyadenylation before being exported for translation. In contrast, prokaryotic mRNAs are often translated while they are still being transcribed, lacking these complex modifications and typically being much shorter-lived than their eukaryotic counterparts.

**68.** (C) Small nuclear RNAs (snRNAs) and associated proteins (snRNPs).
The spliceosome is a massive ribonucleoprotein complex primarily composed of five small nuclear RNAs and numerous associated proteins. These components assemble into units known as small nuclear ribonucleoproteins, or snRNPs. The snRNAs, particularly $U_1$, $U_2$, $U_4$, $U_5$, and $U_6$, play critical roles in recognizing splice sites and catalyzing the two transesterification reactions required to remove introns and ligate exons together.

**69.** (B) An invariant GU
The $5'$ splice site of a typical eukaryotic nuclear intron is defined by a highly conserved sequence, starting with an invariant GU dinucleotide. This sequence is essential for recognition by the $U_1$ snRNP through complementary base pairing. Mutations in these invariant nucleotides often lead to splicing defects, causing the retention of introns or the usage of incorrect, cryptic splice sites during processing.

**70.** (A) The $2'$-OH of the branch point adenosine.
In the first catalytic step of pre-mRNA splicing, the $2'$-OH group of a specific branch point adenosine performs a nucleophilic attack on the $5'$ splice site. This reaction cleaves the phosphodiester bond at the exon-intron boundary and creates a unique $2' - 5'$ linkage between the branch point and the $5'$ end of the intron. This step forms the characteristic lariat intermediate structure.

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**71.** (A) A lariat structure
During the typical spliceosome-mediated splicing reaction, a unique intermediate structure known as a lariat is formed. This occurs when the $5'$ end of the intron is covalently linked to the branch point adenosine via a $2' - 5'$ phosphodiester bond. This lariat contains the entire intron sequence and remains attached to the second exon until the second transesterification step completes the process.

**72.** (D) They catalyze excision without the need for a spliceosome.
Group I introns are characterized by their ability to undergo selF-splicing without the assistance of a large spliceosome complex. They function as catalytic RNAs, or ribozymes, that utilize an exogenous guanosine cofactor to initiate the splicing reaction. The RNA folding provides the necessary active site to catalyze two successive transesterification reactions, resulting in the excision of the intron and ligation.

**73.** (A) Generating protein isoforms from a single gene locus.
The most profound biological impact of alternative splicing is the generation of multiple protein isoforms from a single gene locus. By selectively including or excluding different exons, a cell can produce proteins with distinct functional properties, localization, or stability. This mechanism greatly expands the functional diversity of the proteome in complex eukaryotes, allowing for specialized roles in different tissues or stages.

**74.** (C) Tissue-specific alternative splicing.
Tissue-specific alternative splicing is a major mechanism contributing to the regulation of protein diversity across different cell types. Specialized splicing factors expressed in certain tissues can influence which exons are included in the mature mRNA. This allows the same genetic information to be used to create functionally distinct proteins tailored to the specific needs of the heart, brain, liver, or other organs.

**75.** (B) II only
The genetic code is characterized by several key properties, including high degeneracy and near-universality across all life forms. It is also read continuously without punctuation or gaps. However, the code is strictly non-overlapping, meaning each nucleotide is part of only one codon in a single reading frame. Therefore, the statement claiming the code is overlapping is mathematically and biologically incorrect.

**76.** (A) Multiple codons can code for the same amino acid.
Degeneracy in the genetic code refers to the observation that multiple different codons can specify the same amino acid. This redundancy is primarily found at the third position of the codon, where changes often do not alter the resulting polypeptide. With sixty-one sense codons but only twenty standard amino acids, degeneracy provides a buffer against certain mutations and increases translational efficiency.

**77.** (B) The wobble hypothesis
The wobble hypothesis explains how a single tRNA molecule can recognize and base-pair with multiple synonymous codons. It proposes that the base pairing rules are less stringent at the third position of the codon and the corresponding first position of the anticodon. This flexibility allows for non-standard interactions, reducing the total number of unique tRNA species required by a cell.

**78.** (A) The first position ($5'$ end) of the anticodon
According to the wobble hypothesis, the first position at the $5'$ end of the tRNA anticodon is where non-standard base pairing occurs. This position corresponds to the third base at the $3'$ end of the mRNA codon.

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**79.** (D) The code evolved early and was heavily conserved.
The near-universality of the genetic code implies that the system of translating nucleotide sequences into amino acids evolved early in the history of life and has been heavily conserved. Because the code is essentially the same from bacteria to humans, it suggests a common ancestor for all organisms. The extreme conservation highlights the functional importance of maintaining a stable translation system.

**80.** (C) UGA – Leucine
The genetic code features specific assignments for all sixty-four codons. AUG serves as the start codon and codes for methionine, while UAA and UAG are standard stop signals. UGG is the unique codon for tryptophan. However, UGA is typically a stop codon in the universal code, not an assignment for leucine. This misidentification

initiation of transcription, resulting in a high steady-state level of the corresponding mRNA. The strength of a promoter is a key determinant in regulating the amount of protein produced by a cell, allowing for fine-tuned control of gene activity.

**27.** (D) Coding sequence – Exclusively contains regulatory elements
The components of a transcription unit serve specific roles during RNA synthesis. The promoter binds RNA polymerase, the start site marks the first nucleotide, and the terminator signals dissociation. However, the coding sequence primarily contains the information for protein synthesis rather than exclusively regulatory elements. Regulatory elements like enhancers or silencers are often located outside the boundaries of the main coding sequence.

**28.** (C) Viral genomes like bacteriophage phiX174
Overlapping genes occur most frequently in viral genomes, such as bacteriophage phiX174. In these systems, a single segment of DNA encodes multiple proteins using different reading frames. This arrangement is less common in larger eukaryotic genomes where space is not a limiting factor. The high frequency in viruses reflects their evolutionary need for extreme genomic efficiency within very small physical particles.

**29.** (B) Maximizing information storage in restricted genome sizes
The primary evolutionary driver for overlapping genes in bacteriophages is the need to maximize information storage within restricted genome sizes. Viral capsids have physical limits on the amount of DNA they can package. By using overlapping reading frames, viruses can encode a greater number of essential proteins without increasing their total DNA length, providing a significant advantage for replication and survival.

**30.** (A) By utilizing alternate translation start sites and frames
Overlapping genes typically encode distinct functional proteins from the same DNA segment by utilizing alternate translation start sites and different reading frames. A single nucleotide sequence can be read in three possible frames, each producing a unique polypeptide. This mechanism allows a single transcript or DNA region to harbor multiple sets of genetic instructions, which are independently decoded by the host's ribosomes.

**31.** (D) Evolutionary sequence divergence
The presence of overlapping genes significantly constrains evolutionary sequence divergence. Because a single nucleotide change can simultaneously affect multiple distinct proteins, most mutations are likely to be deleterious to at least one gene product. This dual pressure slows down the accumulation of mutations and limits the rate at which these specific genomic regions can evolve compared to non-overlapping gene segments.

**32.** (A) One sequence can harbor multiple open reading frames.
The phenomenon of overlapping genes in bacteriophage phiX174 demonstrates that a single DNA sequence can harbor multiple open reading frames. By initiating translation at different start codons, the ribosome can produce several distinct proteins from a single shared nucleotide sequence. This discovery highlighted the incredible efficiency of viral genomes and challenged the earlier assumption that each DNA segment encodes only one polypeptide.

**33.** (B) Sigma factor
In prokaryotes, the sigma factor is the subunit of the RNA polymerase holoenzyme responsible for conferring specific promoter recognition. It binds to the $-10$ and $-35$ consensus sequences, ensuring that transcription begins at the correct site. Once the transcription complex has successfully initiated and escaped the promoter, the sigma factor typically dissociates from the core enzyme to allow for elongation.

**34.** (D) Catalyzing the formation of phosphodiester bonds
The beta and beta-prime subunits constitute the catalytic core of the prokaryotic RNA polymerase. Their primary functional role is to facilitate the polymerization of ribonucleotides and catalyze the formation of phosphodiester bonds during RNA synthesis. They also form the DNA-binding channel and the active site where the DNA template is read and the nascent RNA transcript is elongated throughout the process.

**35.** (A) I and III
Sigma 70 is the primary housekeeping factor in Escherichia coli, responsible for transcribing

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reaction between the carboxyl group of the growing polypeptide in the P site and the amino group of the incoming amino acid in the A site. This process is essential for the elongation of the protein chain within the ribosomal large subunit.

**91.** (B) The 23S or 28S ribosomal RNA
Current structural evidence has demonstrated that peptidyl transferase activity is a function of the large ribosomal RNA rather than a protein. In prokaryotes, the 23S rRNA catalyzes the peptide bond formation, while the 28S rRNA performs this role in eukaryotes. This discovery confirms that the ribosome is essentially a ribozyme, highlighting the ancient evolutionary role of RNA in catalysis.

**92.** (A) The ribosome shifts one codon toward the 3′ end of the mRNA.
During the translocation step of translation elongation, the ribosome shifts exactly one codon toward the 3′ end of the mRNA transcript. This movement is powered by the hydrolysis of GTP and involves the relocation of the peptidyl-tRNA from the A site to the P site. Simultaneously, the deacylated tRNA moves to the E site for release, clearing the A site.

**93.** (C) Release factor ($RF_1$ or $RF_2$)
In prokaryotes, stop codons are recognized by specialized proteins known as release factors rather than by tRNAs. $RF_1$ recognizes UAA and UAG, while $RF_2$ identifies UAA and UGA. When one of these factors binds to the A site, it signals that the end of the coding sequence has been reached, initiating the final steps of the translation termination process.

**94.** (D) Hydrolysis of the ester bond linking the polypeptide to the tRNA in the P site
The direct outcome of release factor binding to a stop codon in the A site is the hydrolysis of the ester bond linking the completed polypeptide to the tRNA. This reaction, catalyzed by the peptidyl transferase center, releases the protein from the ribosome. Following this event, the entire translation complex dissociates, allowing the ribosomal subunits to be recycled for another.

**95.** (A) P-2, Q-1, R-4, S-3
Post-translational modifications profoundly affect protein function. Phosphorylation alters activity via kinase action, while ubiquitination targets proteins for proteasomal degradation. Glycosylation modulates folding and cell-surface recognition, and acetylation affects chromatin structure by neutralizing lysine charges. Correctly matching these modifications to their consequences provides a clear overview of how the cell regulates the activity and stability of its diverse proteome.

**96.** (B) Phosphatases
Phosphatases are the class of enzymes specifically responsible for removing phosphate groups from proteins through hydrolysis. This process, known as dephosphorylation, often acts in opposition to kinases to regulate cellular signaling pathways. By switching proteins between phosphorylated and unphosphorylated states, phosphatases help control diverse biological processes, including metabolism, cell division, and the response to external stimuli within the cell.

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**97.** (C) Lysine
The attachment of ubiquitin to a target protein typically occurs on specific lysine residues. This process involves the formation of an isopeptide bond between the C-terminus of ubiquitin and the epsilon-amino group of the lysine. Polyubiquitination at this site creates a signal that is recognized by the 26S proteasome, leading to the efficient degradation of the tagged protein within the.

**98.** (A) Transcriptional activation
The hyperacetylation of core histone proteins is consistently associated with transcriptional activation. Acetyl groups added to lysine residues neutralize their positive charge, weakening the electrostatic interaction between histones and the negatively charged DNA backbone. This results in a more relaxed, open chromatin structure known as euchromatin, which allows easier access for RNA polymerase and the general transcription machinery to the.

**99.** (A) The complex is larger and has a different charge, thus migrating slower.
In an Electrophoretic Mobility Shift Assay, the physical basis for the observed shift is the change in size and charge when a protein binds to DNA. The resulting protein-DNA complex is significantly larger than free DNA and often has a different net charge. Consequently, the complex migrates much slower through the polyacrylamide gel, appearing as a distinct band higher.

**172.** (A) A single mutation could simultaneously alter multiple distinct viral proteins.
The presence of overlapping genes in a viral genome implies that a single mutation could simultaneously alter multiple distinct viral proteins. Because two different reading frames share the same nucleotide sequence, a change in one codon may also change a codon in the second frame. This imposes a strong selective pressure, as most mutations are likely to be harmful.

**173.** (A) The muton is the smallest unit of mutation (a single base pair), while the cistron is the unit of function (a gene).
The fundamental difference between the muton and the cistron, as defined by Benzer, lies in their scale. The muton is the smallest unit of mutation, corresponding to a single base pair. The cistron is the unit of function, representing a whole gene that encodes a polypeptide. This distinction highlights the discovery that genes are divisible entities made of.

**174.** (C) N-linked glycosylation
In eukaryotes, processes like 5′ capping, splicing, and 3′ cleavage occur cotranscriptionally, meaning they happen while the RNA polymerase is still transcribing the gene. However, N-linked glycosylation is a post-translational modification that occurs in the endoplasmic reticulum after the protein has been synthesized and translocated. Therefore, glycosylation is not linked to the transcription process itself in the.

**175.** (A) P-2, Q-4, R-3, S-1
Translational components serve specialized functions during protein synthesis. eIF4E binds the 5′ cap of mRNA, while elongation factors like EF-G mediate ribosomal translocation. Release factors such as $eRF_1$ are responsible for recognizing stop codons, and peptidyl transferase catalyzes the formation of peptide bonds. Correctly matching these factors highlights the coordinated steps required to decode genetic information into functional.

**176.** (A) High intracellular levels of charged tRNA-Trp
High intracellular levels of charged tRNA-Trp directly trigger the attenuation mechanism to terminate transcription in the Trp operon. When tryptophan is abundant, the ribosome does not stall at the tandem Trp codons in the leader sequence. This allows the formation of a terminator hairpin, which signals the RNA polymerase to dissociate before transcribing the structural genes, preventing unnecessary enzyme.

**177.** (B) All biological catalysts (enzymes) are proteins.
The discovery of catalytic RNA challenged the long-held biochemical dogma that all biological catalysts, or enzymes, are proteins. Ribozymes demonstrated that RNA molecules could also possess intrinsic enzymatic activity, such as in the case of selF-splicing introns and the ribosomal peptidyl transferase center. This finding suggested an ancient "RNA world" where RNA served as both the genetic material and.

**178.** (A) To identify all genomic binding sites for a transcription factor or modified histone in vivo
The primary analytical goal of the ChIP-seq technique is to identify all genomic binding sites for a transcription factor or a specific modified histone in vivo. By combining chromatin immunoprecipitation with next-generation sequencing, researchers can create high-resolution maps of protein-DNA interactions across the entire genome. This data is essential for understanding gene regulation, epigenetic states, and the global organization.

**179.** (B) Genes with similar sequences (homologs) likely share similar evolutionary origins and functions.
In silico gene mining relies on the foundational assumption that genes with similar sequences likely share similar evolutionary origins and functions. These homologs often maintain conserved motifs required for their biological activity. By comparing newly sequenced genomes with databases of known genes, researchers can predict the function of novel sequences, facilitating the identification of essential traits and pathways in.

**180.** (B) PSI-BLAST
PSI-BLAST is the database search algorithm that uses a position-specific scoring matrix to detect distant evolutionary relationships. It works by iteratively refining the search profile based on the sequences found in previous rounds. This approach allows it to identify remote homologs that share conserved motifs even if their overall sequence similarity is low, making it more sensitive than standard.

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to the operator sequence, physically preventing RNA polymerase from transcribing the structural genes. This negative control mechanism ensures that the cell does not waste energy producing enzymes when their substrate is unavailable in the surrounding environment.

**110.** (A) It binds the repressor, inducing a conformational change that prevents operator binding.
Allolactose acts as the natural inducer of the Lac operon by binding to the Lac repressor protein. This binding induces a conformational change that significantly reduces the repressor's affinity for the operator DNA. As a result, the repressor dissociates from the operon, clearing the path for RNA polymerase to initiate transcription of the genes required for lactose metabolism and.

**111.** (D) Catabolite repression
Catabolite repression is the phenomenon that explains why Escherichia coli preferentially uses glucose over other carbon sources like lactose. When glucose is available, the cell suppresses the expression of operons involved in the metabolism of alternative sugars. This ensures the most efficient energy source is utilized first, mediated by the intracellular concentration of cyclic AMP and its interaction with.

**112.** (A) Low levels of cyclic AMP (cAMP)
In the mechanism of catabolite repression, high levels of glucose lead to low intracellular concentrations of cyclic AMP. Glucose inhibits the enzyme adenylate cyclase, which is responsible for synthesizing cAMP from ATP. Because cAMP is required for the activation of the Catabolite Activator Protein, its depletion prevents the high-level transcription of operons like Lac, even if their specific.

**113.** (C) Binding of cAMP to the CAP protein
For the Catabolite Activator Protein to bind the Lac promoter and stimulate transcription, it must first bind to cyclic AMP. The cAMP-CAP complex then attaches to a specific site upstream of the promoter, where it recruits RNA polymerase and enhances its binding affinity. This positive control mechanism ensures that the operon is only highly active when glucose levels are.

**114.** (B) Repressible negative control
The Trp operon in Escherichia coli exemplifies repressible negative control. It encodes the enzymes necessary for tryptophan biosynthesis and is active when cellular levels of this amino acid are low. When tryptophan concentrations rise, it acts as a corepressor, binding to the Trp repressor and enabling it to attach to the operator. This blocks transcription, preventing the unnecessary synthesis.

**115.** (B) III only
Transcriptional attenuation in the Trp operon relies on the coupling of transcription and translation and involves alternative mRNA hairpins. High tryptophan levels lead to a terminator structure, stopping synthesis early. However, it is incorrect to say attenuation functions completely independently of the Trp repressor, as both mechanisms work together to provide a robust response to the cell's metabolic tryptophan.

**116.** (A) The ribosome stalls at tandem Trp codons, allowing an anti-terminator hairpin to form.
When tryptophan levels are exceptionally low, the ribosome stalls at tandem Trp codons within the leader peptide sequence. This stalling prevents the formation of the terminator hairpin and instead allows an anti-terminator hairpin to form between regions two and three of the mRNA. As a result, RNA polymerase continues transcribing the full operon, ensuring the production of biosynthetic enzymes.

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**117.** (A) The simultaneous occurrence of transcription and translation
The simultaneous occurrence of transcription and translation is the critical mechanistic factor that enables attenuation as a regulatory strategy. In prokaryotes, ribosomes begin translating the leader mRNA while RNA polymerase is still synthesizing the downstream segments. This coupling allows the position of the ribosome to directly influence the secondary structure of the transcript, determining whether transcription will terminate or.

**118.** (C) Cytosine
Eukaryotic DNA methylation predominantly occurs on the cytosine base, specifically within CpG dinucleotides. The enzyme DNA methyltransferase adds a methyl group to the fifth carbon of the cytosine ring, forming

structural result is increased DNA accessibility. This creates a nucleosome-free region that allows transcription factors and RNA polymerase to bind to their target sequences. By clearing these protein barriers, the cell can effectively transition from a repressed state to active transcription, facilitating the expression of specific genes.

**155.** (A) IV, II, I, III
The Electrophoretic Mobility Shift Assay follows a specific sequential order. First, a DNA probe is radioactively or fluorescently labeled. Next, this probe is incubated with a protein extract to allow binding. The mixture then undergoes electrophoresis on a non-denaturing gel to separate free DNA from protein-DNA complexes. Finally, the labeled DNA fragments are detected to visualize the.

**156.** (A) Alignment of cDNA to the genome showing continuous gaps corresponding to introns
The most definitive evidence of a spliced transcript comes from the alignment of cDNA to the corresponding genomic sequence. This comparison reveals continuous gaps in the cDNA that match the locations of introns in the genome. These gaps demonstrate that the non-coding regions were present in the primary transcript but were removed during the process of RNA splicing.

**157.** (B) The length of its poly-A tail
The structural classification of a transcription factor is based on its DNA-binding motifs, such as zinc fingers or helix-loop-helix domains. These features determine the specificity and affinity of the protein for its target promoter. In contrast, the length of a poly-A tail is a characteristic of mRNA stability rather than the structural properties of a DNA-binding protein.

**158.** (A) Alternative splicing
The production of distinct functional mRNAs from a single gene in different tissues, such as the brain and liver, is best explained by alternative splicing. This process allows the cell to selectively include or exclude exons, resulting in different protein isoforms tailored to specific physiological roles. This mechanism is a key driver of proteomic diversity in complex multicellular organisms.

**159.** (C) It is expressed at a constant level under all conditions.
A strictly constitutive gene is defined by its constant level of expression under all environmental and physiological conditions. These genes, often referred to as housekeeping genes, encode proteins that are essential for basic cellular functions, such as metabolism or structural integrity. Unlike regulated genes, they do not require specific inducers or activators and are transcribed continuously by the cell.

**160.** (A) Tryptophan
Tryptophan serves as the direct co-repressor for the Trp operon in Escherichia coli. When intracellular tryptophan levels are high, the molecule binds to the inactive aporepressor protein. This binding induces a conformational change that enables the repressor to attach to the operator sequence, thereby blocking RNA polymerase and shutting down the transcription of the biosynthetic enzymes in the.

**161.** (A) Repressor dissociates; CAP binds with cAMP to activate transcription
When E. coli experiences glucose depletion and lactose abundance, the regulatory response involves two main steps. First, the absence of glucose leads to high cAMP levels, causing CAP to bind and activate transcription. Simultaneously, the presence of lactose provides the inducer allolactose, which causes the repressor to dissociate from the operator. Together, these events ensure the high-level expression.

**162.** (B) It prevents the enhancer from activating that specific promoter.
The presence of an insulator element placed between an enhancer and a promoter prevents the enhancer from activating that specific promoter. Insulators act as directional barriers that block the interaction between regulatory elements in different chromatin domains. This ensures that a gene is only influenced by its correct enhancers, maintaining the precision of transcriptional control within complex genomic environments.

**163.** (C) The phosphorylated C-terminal domain of RNA polymerase II
The addition of a 7-methylguanosine cap to eukaryotic pre-mRNA is catalyzed by enzymes that associate with the phosphorylated C-terminal domain of RNA polymerase II.

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primary transcript has been synthesized but before translation is complete. By determining which exons are joined or how long a transcript remains functional in the cytoplasm, the cell can fine-tune the types and amounts of proteins produced without changing the transcription.

**128.** (B) It causes a general inhibition of translation initiation by blocking nucleotide exchange.
The phosphorylation of eukaryotic initiation factor 2 causes a global inhibition of cellular translation. This modification occurs in response to various stress conditions and prevents the exchange of GDP for GTP on the factor. Without GTP, $eIF_2$ cannot deliver the initiator tRNA to the small ribosomal subunit, effectively blocking the initiation of new rounds of protein synthesis across the.

**129.** (A) They frequently repress the translation of the downstream main coding sequence.
Upstream open reading frames located in the 5′ untranslated region of eukaryotic mRNAs frequently repress the translation of the main coding sequence. When a ribosome encounters a uORF, it may initiate translation and then dissociate after finishing the short peptide, failing to reach the primary start codon. This mechanism allows for the precise translational control of genes involved in.

**130.** (B) The 5′ cap and the 3′ poly-A tail
The stability of a eukaryotic mRNA transcript is heavily dependent on the integrity of the 5′ cap and the 3′ poly-A tail. These structures work together to protect the RNA from exonucleases and are often bridged by proteins to form a circularized complex. The removal of either the cap or the tail typically serves as a signal.

**131.** (A) Nonsense-mediated decay (NMD)
Nonsense-mediated decay is the intracellular pathway responsible for degrading mRNAs that contain premature stop codons. This surveillance mechanism identifies transcripts where translation would terminate early, preventing the production of potentially harmful truncated proteins. By linking splicing markers to the position of the stop codon, the cell can distinguish between legitimate termination sites and those resulting from mutations or errors.

**132.** (A) Using conserved sequence motifs from known genes to design primers or probes to isolate related genes
Homology-based cloning relies on using conserved sequence motifs from known genes to design primers or probes for isolating related genes. This strategy assumes that essential functional domains are evolutionary conserved across different species. By targeting these similarities, researchers can identify and clone homologous genes in unsequenced organisms, facilitating the study of gene families and the discovery of novel genetic.

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**133.** (A) BLAST (Basic Local Alignment Search Tool)
BLAST is the fundamental standard algorithm for local sequence alignment in bioinformatics. it allows researchers to compare a query sequence against a massive database to find regions of high similarity. By using a heuristic approach, BLAST rapidly identifies potential homologs and provides statistical evidence of their relationship, making it an indispensable tool for gene discovery, functional annotation, and evolutionary.

**134.** (B) They are highly effective for creating probabilistic profiles of protein families and identifying remote homologs.
Hidden Markov Models are highly effective for creating probabilistic profiles of protein families and identifying remote homologs. Unlike simple sequence alignments, HMMs can capture complex patterns of conservation and variation across a whole family of related proteins. This makes them exceptionally powerful for predicting the function of newly discovered sequences that share only distant evolutionary ties with known.

**135.** (A) P-3, Q-4, R-2, S-1
Molecular analysis techniques are essential for genome studies. In silico identification involves the computational discovery of novel genes, while homology alignment compares sequences for evolutionary conservation. ChIP-seq maps protein binding sites across the entire genome, and transcriptomics profiles all expressed RNAs. Matching these concepts highlights the integrated approach required to understand the structure, function, and regulation of complex.

**100.** (B) The specific DNA sequence bound by a protein
DNase I footprinting is a technique designed to identify the specific DNA sequence bound by a protein. When a protein attaches to a DNA fragment, it protects that region from digestion by the DNase I enzyme. By comparing the cleavage patterns of DNA with and without the protein, researchers can pinpoint the exact binding site, which appears as a gap.

**101.** (D) DNase I randomly cleaves unprotected DNA but cannot cut where a protein is tightly bound.
The fundamental principle of DNase I footprinting is that the enzyme randomly cleaves unprotected DNA but cannot cut where a protein is tightly bound. The protein acts as a physical shield, preventing the endonuclease from accessing the phosphodiester backbone. This creates a "footprint" of protected fragments when the samples are analyzed by electrophoresis, revealing the location of the DNA-binding.

**102.** (B) The association of specific proteins with specific genomic regions
Chromatin immunoprecipitation is a powerful technique used to analyze the association of specific proteins with particular genomic regions in vivo. It allows researchers to map the binding sites of transcription factors, histones, or other DNA-associated proteins across the entire genome. By providing a snapshot of protein-DNA interactions within live cells, ChIP is essential for understanding gene regulatory networks and chromatin.

**103.** (B) Crosslinking of proteins to DNA using formaldehyde
In a standard ChIP assay, the first step performed on live cells is the crosslinking of proteins to DNA using formaldehyde. This chemical treatment creates covalent bonds that freeze protein-DNA interactions in their native state. Following crosslinking, the chromatin is fragmented, and specific antibodies are used to isolate the protein of interest along with its associated DNA sequences for further.

**104.** (A) Protein-protein interactions
The yeast two-hybrid system is an analytical technique used primarily to investigate protein-protein interactions. It relies on the ability of two proteins to bring together two halves of a split transcription factor, thereby activating a reporter gene. This system allows for the large-scale screening of potential interaction partners within a cellular environment, helping to map complex protein networks and signaling.

**105.** (D) I, II and III
The yeast two-hybrid system relies on the modular nature of eukaryotic transcription factors like GAL4. In this assay, the "bait" protein is fused to a DNA-binding domain, while the "prey" protein is fused to an activation domain. If the two proteins interact, the domains are reunited to drive transcription. All three statements accurately describe the fundamental components and mechanism.

**106.** (A) Transcription requires the binding of an activator protein to the promoter region.
In prokaryotic gene regulation, a positive control system is defined by the requirement of an activator protein to initiate transcription. When the activator binds to a specific site near the promoter, it facilitates the recruitment or activity of RNA polymerase. Without the binding of this regulatory protein, the operon remains at a basal level or is completely switched off, ensuring.

**107.** (C) It is usually on and is turned off when a specific corepressor is present.
A repressible operon system is characterized by being usually on, meaning transcription occurs constitutively. It is only turned off when a specific corepressor molecule is present in the cell. The corepressor binds to an aporepressor protein, enabling it to attach to the operator and block RNA polymerase. This mechanism allows the cell to stop production when end products are abundant.

**108.** (A) Inducible
The Lac operon of Escherichia coli is a classic example of an inducible regulatory system. It is normally repressed by the LacI protein and only becomes active when an inducer, such as allolactose, is present. The inducer binds to the repressor, causing it to release the operator and allowing transcription to proceed. This ensures that enzymes for lactose metabolism are.

**109.** (D) It functions as an allosteric repressor binding to the operator.
The LacI protein functions as an allosteric repressor that regulates the expression of the Lac operon. In the absence of lactose, it binds tightly

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**145.** (D) I, II, III, IV and V
The assembly of the spliceosome involves a series of sequential snRNP interactions. $U_1$ binds the 5′ site, followed by $U_2$ at the branch point. The tri-snRNP complex then enters, leading to the expulsion of $U_1$ and $U_4$. This process creates a catalytic core dominated by snRNAs. All five statements accurately describe the complex structural and functional steps of splicing.

**146.** (A) The precise fit of the amino acid in the activation site and a separate editing site
To ensure translational fidelity, aminoacyl-tRNA synthetases utilize a two-step verification process. The precise fit of the amino acid in the activation site provides the first level of specificity. Furthermore, a separate editing site identifies and deletes incorrectly charged amino acids that are too small or chemically similar. This dual-site mechanism prevents the attachment of the wrong amino acid to.

**147.** (A) $eEF_1A$ (equivalent to prokaryotic EF-Tu)
In eukaryotes, the elongation factor $eEF_1A$ is responsible for delivering the aminoacyl-tRNA to the ribosomal A site. This factor acts in a GTP-dependent manner, forming a ternary complex with the tRNA. Once the correct codon-anticodon match is made, GTP is hydrolyzed, and $eEF_1A$ dissociates, allowing the ribosome to proceed with peptide bond formation and subsequent elongation steps.

**148.** (B) GTP
GTP is the specific energetic molecule hydrolyzed during the delivery of an aminoacyl-tRNA to the ribosome. Elongation factors, such as EF-Tu in prokaryotes or $eEF_1A$ in eukaryotes, use the energy from GTP hydrolysis to ensure the accuracy and speed of the decoding process. This hydrolysis serves as a critical checkpoint, allowing for the rejection of incorrect tRNA molecules.

**149.** (B) Stalling of ribosomes at stop codons without chain release
A mutation that eliminates the function of eukaryotic Release Factor 1 would immediately lead to the stalling of ribosomes at stop codons. $eRF_1$ is essential for recognizing stop signals and triggering the release of the completed polypeptide chain. Without functional $eRF_1$, the translation complex cannot dissociate, preventing the termination of protein synthesis and the recycling of ribosomal.

**150.** (B) N-linked glycosylation
N-linked glycosylation is a post-translational modification characterized by the addition of complex carbohydrate chains to specific asparagine residues. This process occurs within the context of the consensus sequence Asn-X-Ser/Thr. The attachment of these oligosaccharides is crucial for correct protein folding, stability, and cell-surface recognition, playing a vital role in the maturation of secreted and membrane-bound proteins.

**151.** (C) The rough endoplasmic reticulum
N-linked glycosylation of newly synthesized proteins predominantly initiates within the lumen of the rough endoplasmic reticulum. As the polypeptide is translocated into the ER, a pre-assembled oligosaccharide is transferred to specific asparagine residues. This initial modification is further refined as the protein moves through the Golgi apparatus, where additional sugars are added or removed to form the final.

**152.** (A) The suppression of transcription by the Lac repressor binding the operator
Negative control in the lac operon is best illustrated by the suppression of transcription by the Lac repressor. When lactose is absent, the repressor binds tightly to the operator site, physically blocking RNA polymerase from accessing the promoter. This mechanism ensures that the genes for lactose metabolism are only expressed when the substrate is available, preventing the unnecessary expenditure.

**153.** (A) Constitutive expression of the lac operon
A mutation in the lac operator, known as lacOc, prevents the Lac repressor from binding to the DNA. This results in the constitutive expression of the lac operon, where the structural genes are transcribed regardless of whether lactose is present or absent. This phenotype demonstrates the importance of the operator-repressor interaction in maintaining proper negative regulation of the.

**154.** (B) Transcription factors and RNA polymerase can access the DNA.
When chromatin remodeling complexes push nucleosomes away from a promoter, the primary

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**136.** (B) Orthologs
Orthologs are genes in different species that evolved from a common ancestral gene via speciation. They typically retain the same function in the new organisms. Identifying orthologous relationships is critical for evolutionary studies and for transferring functional knowledge from well-studied model organisms to other species. They represent the direct lineage of a gene across the diversity of life forms.

**137.** (C) Through gene duplication events
Paralogous genes arise within a single genome through gene duplication events. Once a gene is duplicated, the two copies can evolve independently, often leading to new functions or specialized roles within the organism. Paralogs constitute multigene families and contribute to the expansion of the genetic repertoire, providing the raw material for evolutionary innovation and the development of complex biological.

**138.** (A) Single Nucleotide Polymorphism (SNP) databases and GWAS
The identification of novel alleles for agronomically important traits heavily utilizes Single Nucleotide Polymorphism databases and Genome-Wide Association Studies. These resources allow researchers to link specific genetic variations with desirable phenotypes in crop plants. By analyzing large populations, breeders can pinpoint alleles that contribute to disease resistance, yield, or stress tolerance, facilitating the development of improved agricultural varieties.

**139.** (A) Whole-genome Next Generation Sequencing (NGS)
Whole-genome Next Generation Sequencing provides the highest resolution for determining the global complexity of a newly discovered plant genome. Unlike earlier methods, NGS allows for the comprehensive identification of all coding and non-coding sequences, including repetitive elements. This detailed data enables researchers to assemble the genome, map gene locations, and analyze structural variations with unprecedented precision and scale.

**140.** (C) The massive abundance of highly repetitive DNA and long introns
The massive abundance of highly repetitive DNA and long introns introduces the most significant challenge when computationally annotating a large eukaryotic genome. These features can obscure the boundaries of structural genes and make it difficult for algorithms to accurately predict open reading frames. Differentiating between functional exons and interspersed repeats requires sophisticated modeling and additional experimental data like RNA-seq for.

**141.** (C) An AT-rich consensus sequence that binds the ORC
The identification of an origin of replication in yeast, known as an Autonomously Replicating Sequence, relies on a specific AT-rich consensus sequence. This motif is recognized and bound by the Origin Recognition Complex, which serves as the scaffold for recruiting other replication proteins. The high AT content facilitates the localized unwinding of the DNA, allowing replication to begin.

**142.** (A) The Initiator (Inr) element
A genomic region that lacks a TATA box but initiates transcription from a focused start point typically relies on the Initiator element. The Inr sequence encompasses the start site and is directly recognized by subunits of the TFIID complex. This interaction allows for the precise positioning of the transcription machinery even in the absence of other common core promoter.

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**143.** (B) Its phosphorylation state coordinates transcription elongation and RNA processing events.
The Carboxy-Terminal Domain of RNA polymerase II plays a crucial role in coordinating transcription and RNA processing. Its phosphorylation state changes as the enzyme moves from initiation to elongation and termination. These specific modifications serve as a scaffold for recruiting capping, splicing, and polyadenylation factors, ensuring that the nascent RNA is correctly processed while it is still being synthesized.

**144.** (A) $U_1$
$U_1$ snRNP is the molecular entity that initially recognizes and binds to the 5′ splice site during spliceosome assembly. This interaction is mediated by the complementary base pairing between the $U_1$ snRNA and the conserved sequence at the exon-intron boundary. This binding is the first committed step in splicing, serving to define the start of the intron for.

This association occurs very early during the transcription process, typically when the nascent RNA is only twenty to thirty nucleotides long. This spatial proximity ensures that the $5'$ end is modified and protected immediately.

**164.** (C) It is debranched and rapidly degraded by nuclear ribonucleases.
Immediately after its release from the spliceosome, the lariat intron is targeted for rapid degradation. A specialized debranching enzyme breaks the unique $2' - 5'$ phosphodiester bond at the branch point, converting the lariat into a linear RNA molecule. This linear transcript is then digested by nuclear ribonucleases, ensuring that the non-coding sequences are recycled and do not accumulate.

**165.** (A) Both A and R are true and R is the correct explanation of A.
The genetic code is degenerate, meaning most amino acids are specified by more than one codon. This is mathematically supported by the wobble hypothesis, which allows a single tRNA anticodon to base-pair with multiple synonymous codons. This flexibility typically occurs at the third nucleotide position of the codon, ensuring that various triplets can be translated into the same amino.

**166.** (A) Eukaryotic initiation generally relies on ribosomal scanning from the $5'$ cap, while prokaryotic relies on internal Shine-Dalgarno binding.
Eukaryotic initiation generally relies on ribosomal scanning from the $5'$ cap, while prokaryotic relies on internal Shine-Dalgarno binding. This critical difference in translation initiation is the method of ribosome recruitment. Eukaryotic initiation generally involves the small ribosomal subunit scanning from $5'$ the cap to find the first start codon. In contrast, prokaryotic ribosomes rely on internal binding to the Shine-Dalgarno sequence, which positions the subunit directly at the start site. this allows prokaryotes to initiate.

**167.** (B) Covalent modifications of histone tails serve as docking sites for chromatin-modifying effector proteins.
The histone code hypothesis proposes that covalent modifications of histone tails serve as docking sites for specific chromatin-modifying effector proteins. These modifications, such as acetylation or methylation, create a complex regulatory language that is "read" by other proteins to determine the local chromatin state. This mechanism controls whether genes in a particular region are expressed or silenced in.

**168.** (B) Trimethylation of Histone $H_3$ at Lysine 9 ($H_3K_9me_3$)
The trimethylation of Histone $H_3$ at Lysine 9 is a classic hallmark of transcriptionally inactive heterochromatin. This specific modification provides a binding site for Heterochromatin Protein 1, which promotes chromatin condensation and the spreading of a silenced state. Genes located within these regions are tightly packed and inaccessible to the transcription machinery, ensuring long-term silencing of repetitive elements and.

**169.** (A) The transactivation domain
The transactivation domain is the essential part of a eukaryotic transcription factor that recruits coactivators and chromatin remodeling complexes. While the DNA-binding domain ensures the factor targets the correct gene, the transactivation domain interacts with other proteins to facilitate the assembly of the transcription machinery. By recruiting remodelers, these factors can open up chromatin, allowing for successful gene activation.

**170.** (C) Extensive methylation of the core promoter
Extensive methylation of the core promoter is a mechanism of transcriptional silencing rather than post-transcriptional regulation. It prevents the initiation of transcription by blocking the binding of RNA polymerase. In contrast, mechanisms like miRNA binding, RISC-mediated cleavage, and nonsense-mediated decay occur after the RNA has been synthesized, representing various forms of post-transcriptional control that affect the stability or translation.

**171.** (B) RNA splicing
The significant discrepancy between the length of a primary transcript and the mature mRNA is overwhelmingly due to RNA splicing. Eukaryotic genes often contain long non-coding regions called introns that are removed during processing. Only the coding exons are joined together to form the final functional mRNA. This process can reduce the total length of the transcript by thousands.

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5-methylcytosine. This epigenetic modification is a key regulator of gene expression, often associated with stable long-term silencing and the maintenance of genomic stability throughout the life of the.

**119.** (C) Transcriptional silencing
DNA methylation at CpG islands situated in eukaryotic promoter regions is generally associated with transcriptional silencing. The presence of methyl groups can physically block the binding of transcription factors or recruit specialized proteins that induce a repressive chromatin state. Consequently, genes with heavily methylated promoters are typically inactive, providing a mechanism for tissue-specific gene regulation and the suppression of transposable.

**120.** (A) The histone code
The histone code hypothesis suggests that specific combinations of post-translational modifications on histone tails dictate distinct functional outcomes for chromatin. Modifications like acetylation, methylation, and phosphorylation act as signals that are recognized by effector proteins. These interactions determine whether a particular genomic region will be transcriptionally active or silent, providing a sophisticated layer of epigenetic control over the genetic.

**121.** (B) Hydrolyze ATP to slide, eject, or restructure nucleosomes
ATP-dependent chromatin remodeling complexes use the energy from ATP hydrolysis to physically alter the structure of nucleosomes. They can slide nucleosomes along the DNA, eject them entirely, or restructure them to change DNA accessibility. By repositioning these protein complexes, remodelers allow transcription factors and RNA polymerase to reach promoters, thereby playing a crucial role in the activation and regulation.

**122.** (A) Small double-stranded RNA molecules
Small double-stranded RNA molecules are the central molecular entities in the mechanism of RNA interference. These short RNAs, typically twenty-one to twenty-three nucleotides long, serve as guides for the RNA-induced silencing complex. By base-pairing with complementary messenger RNAs, they target them for degradation or translational repression. This pathway is a fundamental tool for gene regulation and defense against viral.

**123.** (B) Dicer
Dicer is the enzyme responsible for cleaving long double-stranded RNA or pre-miRNA into short fragments during the RNAi pathway. It acts as an RNase III-like protein that measures and cuts the RNA into characteristic twenty-one to twenty-three nucleotide lengths. These small fragments, known as siRNAs or miRNAs, are then loaded into the silencing complex to initiate targeted gene.

**124.** (B) The Argonaute protein
The Argonaute protein serves as the primary functional core of the RNA-induced silencing complex. It binds the guide strand of the small RNA and uses it to identify complementary target mRNAs. Depending on the degree of complementarity, Argonaute can either cleave the transcript directly through its endonuclease activity or recruit additional factors to repress translation and promote mRNA decay.

**125.** (A) Both A and R are true and R is the correct explanation of A.
MicroRNAs can inhibit translation without perfectly pairing with the target mRNA. While perfect sequence complementarity is often required for Argonaute-mediated transcript cleavage, partial pairing, especially in the 3′ untranslated region, typically leads to translational repression. This flexibility allows a single miRNA to regulate multiple different genes, enhancing the complexity and reach of the cell's post-transcriptional regulatory network.

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**126.** (A) siRNAs are usually derived from exogenous long dsRNA, whereas miRNAs are encoded by endogenous cellular genes.
siRNAs and miRNAs differ primarily in their cellular biogenesis and sources. Small interfering RNAs are usually derived from exogenous long double-stranded RNAs, such as those from viruses or experimental introduction. In contrast, microRNAs are encoded by endogenous cellular genes and processed from specific hairpin precursors. While both use the Dicer and RISC machinery, their origins reflect different biological.

**127.** (A) Alternative splicing and mRNA stability control
Alternative splicing and mRNA stability control are prime examples of post-transcriptional gene regulation. These processes occur after the

**181.** (D) Acting as the primary peptidyl transferase
The 16S rRNA is a vital component of the prokaryotic 30S ribosomal subunit. It provides structural integrity, base-pairs with the Shine-Dalgarno sequence, and stabilizes correct codon-anticodon pairing. However, it does not act as the primary peptidyl transferase. That catalytic function is performed by the 23S rRNA in the 50S subunit, highlighting the specialized roles of different ribosomal RNAs.

**182.** (B) The production of a truncated polypeptide
A nonsense mutation in the middle of a structural gene results in the production of a truncated polypeptide. This occurs because a sense codon is changed into a stop codon, causing translation to terminate prematurely. These shortened proteins are often non-functional or unstable and are frequently targeted for degradation by the cell's surveillance mechanisms, such as nonsense-mediated mRNA.

**183.** (B) The 5′ cap protects the transcript from 3′ to 5′ exonucleases.
Several factors influence eukaryotic mRNA stability, including the poly-A tail and AU-rich elements. Shortening of the tail often initiates degradation, and decapping enzymes act later in the pathway. However, the statement that the 5′ cap protects the transcript from 3′ to 5′ exonucleases is incorrect. The cap specifically protects the 5′ end from 5′ to 3′ degradation.

**184.** (B) The degree of base-pairing complementarity between the miRNA and the mRNA
The degree of base-pairing complementarity between the miRNA and its target mRNA determines whether the transcript is cleaved or translationally repressed. High complementarity usually leads to Argonaute-mediated cleavage, while imperfect pairing, which is more common in animals, typically results in the inhibition of translation. This distinction allows microRNAs to fine-tune gene expression through different molecular mechanisms depending on the.

**185.** (B) II only
Post-translational modifications are key regulators of protein function. Phosphorylation is generally reversible, and glycosylation aids in folding. Acetylation neutralizes lysine charges, and kinases and phosphatases manage phosphate groups. However, the statement that ubiquitination invariably activates the target protein is incorrect. Ubiquitination most often targets proteins for degradation, although it can also serve non-proteolytic regulatory roles in some.

**186.** (A) $eIF_2$ complexed with GTP
During the formation of the eukaryotic 43S pre-initiation complex, the $eIF_2$ complexed with GTP is responsible for bringing the initiator tRNA to the small ribosomal subunit. This factor specifically recognizes the methionyl-tRNA and facilitates its binding to the P site. This step is a prerequisite for the ribosome to begin scanning the mRNA for the appropriate start.

**187.** (B) The presence of common specific cis-regulatory elements (e.g., response elements) in their promoters
In eukaryotes, the coordinated regulation of multiple genes scattered across different chromosomes is achieved through the presence of common specific cis-regulatory elements in their promoters. These "response elements" are recognized by the same set of transcription factors. When a specific signal activates these factors, they bind to all the corresponding genes simultaneously, ensuring a synchronized physiological or metabolic.

**188.** (A) A silencer is a DNA sequence, whereas a corepressor is a protein.
The primary characteristic that differentiates a silencer from a standard corepressor is their molecular nature. A silencer is a specific DNA sequence that serves as a regulatory element, whereas a corepressor is a protein that binds to other transcription factors to inhibit gene expression. Silencers act as the binding sites for the proteins that recruit corepressor complexes to.

**189.** (A) Zinc finger
A DNA-binding protein containing an alpha helix and a beta sheet held together by a single structurally critical heavy metal ion is a zinc finger. The zinc ion coordinates with cysteine and histidine residues to stabilize the fold, allowing the alpha helix to insert into the major groove of the DNA. This motif is widely found in eukaryotic transcription.

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**208.** (C) Degraded, leaving the "guide strand" active within RISC
During the RNA interference pathway, the double-stranded RNA intermediate is unwound, and one strand is selected as the "guide strand." The other strand, known as the "passenger strand," is typically degraded. The guide strand remains associated with the Argonaute protein in the RISC complex, where it provides the specificity needed to target and silence complementary messenger RNA transcripts.

**209.** (D) Promoters determine the exact start site and basal direction of transcription; enhancers modulate the rate independent of position or orientation.
The fundamental difference between an enhancer and a promoter lies in their functional scope. Promoters determine the exact start site and basal direction of transcription, serving as the primary assembly point for the transcription machinery. In contrast, enhancers modulate the rate of transcription independent of their position or orientation. Enhancers can be located very far from the target gene.

**210.** (C) The recruitment of methyl-CpG-binding domain (MBD) proteins and subsequent gene silencing
Extensive DNA methylation at a specific gene locus typically leads to the recruitment of methyl-CpG-binding domain proteins. These proteins then attract additional corepressors and histone deacetylases, resulting in the formation of repressive heterochromatin and subsequent gene silencing. This epigenetic modification is a powerful mechanism for the long-term suppression of gene activity, ensuring stable patterns of gene expression across cell.

**211.** (A) High levels of histone acetylation
High levels of histone acetylation are universally viewed as a hallmark of transcriptionally active chromatin, or euchromatin. This modification, catalyzed by histone acetyltransferases, reduces the positive charge on histone tails, loosening their interaction with DNA. The resulting open chromatin structure is more accessible to RNA polymerase and transcription factors, facilitating the high-level expression of genes within these genomic regions.

**212.** (D) It presents a distinct pattern of hydrogen bond donors, acceptors, and hydrophobic patches unique to each base pair combination.
Most sequence-specific transcription factors prefer binding to the DNA major groove because it presents a distinct pattern of hydrogen bond donors, acceptors, and hydrophobic patches. These chemical signatures are unique to each possible base pair combination, allowing proteins to identify their target sequences with high precision. The wider dimensions of the major groove also accommodate the insertion of.

**213.** (B) Multiple metabolically related genes are transcribed as a single polycistronic mRNA unit.
The structural organization of a prokaryotic operon ensures that multiple metabolically related genes are transcribed as a single polycistronic mRNA unit. This arrangement allows the cell to coordinately regulate the expression of all enzymes in a particular pathway using a single promoter and operator. Consequently, the production of these proteins is synchronized, maximizing efficiency and metabolic responsiveness to.

**214.** (B) The binding of the LacI repressor to the operator
Allolactose disrupts the specific molecular interaction between the LacI repressor and the operator sequence in the lac operon. By binding to the repressor, allolactose induces a conformational change that lowers its affinity for the DNA. This causes the repressor to dissociate, allowing RNA polymerase to proceed with transcription. This induction mechanism is fundamental for responding to the presence.

**215.** (B) Low levels of cAMP prevent CAP from effectively binding and assisting RNA polymerase.
Transcription of the lac operon is limited when both glucose and lactose are present because low levels of cAMP prevent CAP from effectively binding. Glucose availability suppresses adenylate cyclase, reducing the intracellular concentration of cAMP. Without the cAMP-CAP complex to assist RNA polymerase at the promoter, the operon remains at a basal level of expression, prioritizing the metabolism.

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**199.** (A) They are generally longer, interspersed throughout the genome, and may encode reverse transcriptase.
LINEs are moderately repetitive DNA sequences that are generally longer than satellite DNA and interspersed throughout the genome. Unlike the short tandem repeats found in centromeres, LINEs are autonomous retrotransposons that may encode functional proteins such as reverse transcriptase. This allows them to replicate and move to new locations, contributing to genomic diversity and the expansion of the eukaryotic.

**200.** (A) Both A and R are true and R is the correct explanation of A.
Alternative splicing increases the coding capacity of a eukaryotic genome by allowing a single pre-mRNA to be processed into multiple distinct mature mRNAs. This results in the production of different protein isoforms with specialized functions. Consequently, the organism can achieve greater proteomic diversity and complexity without needing to increase the total number of genes, enhancing physiological and evolutionary.

**201.** (A) The consensus sequence AAUAAA
The consensus sequence AAUAAA directs the precise cleavage of a eukaryotic transcript prior to polyadenylation. This motif is recognized by a complex of proteins that cut the RNA approximately ten to thirty nucleotides downstream. The newly generated 3′ end then serves as the substrate for poly-A polymerase, which adds the protective tail necessary for mRNA stability and efficient translation.

**202.** (B) The sequence is under strong negative (purifying) selection due to essential functionality.
If a genomic sequence shows extreme conservation across divergent species, it is most likely under strong negative selection due to essential functionality. Any mutations in such regions are typically harmful and are eliminated from the population. High conservation suggests that the sequence performs a critical role, such as encoding an essential protein domain or serving as a vital regulatory.

**203.** (A) The absence of introns and presence of a 5′ cap and 3′ poly-A tail
A mature eukaryotic mRNA is distinguished from its primary pre-mRNA transcript by several key modifications. Mature mRNA lacks introns, which are removed during splicing, and possesses a 5′ cap and a 3′ poly-A tail. These changes are essential for the transcript's stability, its successful export from the nucleus, and its efficient translation by ribosomes in the cytoplasm of.

**204.** (A) Complementary base pairing with the 5′ splice site consensus sequence
The small nuclear RNA $U_1$ recognizes the 5′ splice site through complementary base pairing with the conserved consensus sequence at the intron boundary. This interaction is the first step in spliceosome assembly and ensures that the boundaries of the intron are accurately identified. The specificity of this pairing is critical for the precise removal of non-coding sequences during RNA processing.

**205.** (A) II, IV, III, I
The initiation of translation in Escherichia coli follows a defined biological sequence. First, initiation factors bind to the 30S ribosomal subunit. The subunit then binds to the mRNA via the Shine-Dalgarno sequence. Subsequently, the initiator tRNA attaches to the start codon at the P site. Finally, the 50S subunit joins the complex, accompanied by GTP hydrolysis and the.

**206.** (A) The localized melting of approximately 14 base pairs of DNA around the start site
The transition from a closed to an open complex during prokaryotic transcription is defined by the localized melting of approximately fourteen base pairs of DNA. This unwinding occurs around the transcription start site, creating a transcription bubble that exposes the template strand. This structural change is a prerequisite for RNA polymerase to begin the synthesis of the nascent RNA molecule.

**207.** (A) They bind to specific mRNAs and guide the RISC complex to cleave them.
Small interfering RNAs primarily function by guiding the RNA-induced silencing complex to cleave specific target mRNAs. Upon binding to a perfectly complementary sequence, the Argonaute protein within the RISC complex cuts the transcript, leading to its rapid degradation. This mechanism provides a highly specific and efficient way to downregulate gene expression and defend the cell against viral RNA infections.

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different amino acid sequence, highlighting the critical role of AUG in genetic.

**243.** (A) Its ability to continuously synthesize RNA without dissociating from the DNA template
The processivity of prokaryotic RNA polymerase is defined as its ability to continuously synthesize RNA without dissociating from the DNA template. High processivity is essential for the production of long transcripts, ensuring that the enzyme remains engaged until it reaches a termination signal. This characteristic is achieved through the stable formation of the transcription bubble and the enzyme's tight.

**244.** (D) $3' - TAGCAT - 5'$
If an mRNA sequence reads $5' - AUCGUA - 3'$, the DNA template strand must be complementary and anti-parallel. Therefore, the template sequence is $3' - TAGCAT - 5'$. During transcription, RNA polymerase reads the template in the $3'$ to $5'$ direction and synthesizes the RNA in the $5'$ to $3'$ direction, substituting uracil for thymine. Correct identification of the template sequence is fundamental for genetic.

**245.** (A) A diffusible regulatory protein that can act on target genes located anywhere in the genome
A trans-acting regulatory element is defined as a diffusible regulatory protein that can act on target genes located anywhere in the genome. These factors, such as transcription activators or repressors, are produced from one gene and travel through the cell to bind to specific DNA sequences elsewhere. Their ability to influence distant genes distinguishes them from cis-acting elements.

**246.** (A) It requires a continuous DNA backbone to exert topological or physical influence on a nearby promoter.
A cis-acting element is restricted to regulating only closely linked genes because it requires a continuous DNA backbone to exert its influence. These elements, such as promoters and enhancers, function by being physically located on the same DNA molecule as the gene they control. This proximity is necessary for the element to facilitate the assembly of the transcription machinery.

**247.** (B) The substituted amino acid may have similar biochemical properties (conservative substitution).
A missense mutation may not entirely abolish a protein's function if the substituted amino acid has similar biochemical properties to the original one. This is known as a conservative substitution. If the new amino acid is chemically related, such as replacing one hydrophobic residue with another, the protein's overall fold and activity might be preserved, minimizing the impact of the.

**248.** (A) It assumes an inactive conformation and cannot bind the operator DNA.
When tryptophan is absent, the Trp aporepressor assumes an inactive conformation and cannot bind to the operator DNA. In this state, the repressor lacks the required structure to attach to the operon, allowing RNA polymerase to initiate transcription of the biosynthetic genes. This ensures that the cell can produce tryptophan when internal levels are insufficient to meet metabolic.

**249.** (A) RNA polymerase can initiate de novo strand synthesis without a primer.
A characteristic that effectively distinguishes RNA polymerase from DNA polymerase is its ability to initiate de novo strand synthesis without a primer. While DNA polymerase requires a pre-existing 3'-OH group to add new nucleotides, RNA polymerase can begin RNA synthesis directly from the DNA template. this capability is fundamental for the initiation of transcription and the subsequent production of.

**250.** (B) P-2, Q-4, R-1, S-3
Core promoter elements serve as specific binding sites for the transcription machinery. The TATA box binds TBP, while the Initiator encompasses the start site. The BRE is located upstream and binds TFIIB, and the DPE is found downstream, often in TATA-less promoters. Correctly matching these motifs to their descriptions highlights the structural diversity and functional specialization of eukaryotic promoter regions.

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**251.** (A) Addition of a lipid anchor (e.g., myristoylation or farnesylation)
The addition of a lipid anchor, such as myristoylation or farnesylation, is the post-translational modification primarily known for anchoring specific proteins to the cytosolic

**216.** (C) By the speed at which the ribosome translates tandem Trp codons in the leader peptide sequence
Trp operon attenuation senses the cellular concentration of tryptophan by monitoring the speed at which the ribosome translates tandem Trp codons in the leader peptide. If tryptophan is abundant, the ribosome moves quickly, allowing the formation of a terminator hairpin. If levels are low, the ribosome stalls, leading to an anti-terminator structure that permits full transcription of the.

**217.** (A) Missense mutation
A genetic mutation that converts a UAG stop codon to a UAC tyrosine codon is classified as a missense mutation. This change results in the incorporation of an amino acid where translation would normally have terminated. By replacing a stop signal with a coding codon, the mutation can lead to the production of an abnormally long and potentially non-functional.

**218.** (A) The specific wobble base pairing allowed at the third position of the codon
The redundancy of the genetic code is primarily accommodated by the specific wobble base pairing allowed at the third position of the codon. This flexibility enables a single tRNA anticodon to recognize multiple synonymous codons, which often vary only at the final nucleotide. Consequently, the cell requires fewer unique tRNA molecules to accurately translate the sixty-one sense codons into.

**219.** (A) X-ray crystallography data interpreted by Watson, Crick, and Franklin
The earliest definitive evidence that DNA is a double-stranded, anti-parallel helix came from X-ray crystallography data interpreted by Watson, Crick, and Franklin. The characteristic "X" pattern observed in B-form DNA diffraction photographs provided the mathematical basis for determining the helical nature and dimensions of the molecule. This discovery was a landmark event that established the physical structure.

**220.** (D) Genome size has absolutely no effect on the Cot curve profile.
DNA reassociation kinetics classify sequences based on their repetition frequency. Highly repetitive sequences renature almost instantly, while unique DNA takes the longest. Moderately repetitive fractions include functional multigene families. However, the statement that genome size has no effect on the *Cot* curve profile is incorrect. Larger genomes with more unique sequences shift the curve toward higher values, reflecting their.

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**221.** (A) Novel alleles and multigene families
The phenomenon of gene duplication followed by divergence is a primary evolutionary source of novel alleles and multigene families. After a duplication event, one copy of the gene can maintain the original function, while the other is free to accumulate mutations and acquire new roles. This process is a major driver of genomic innovation, leading to the development of.

**222.** (C) The E-value (Expect value) in a BLAST search
The E-value is the statistical tool used in BLAST searches to evaluate the probability that a sequence alignment occurred by random chance. A lower E-value indicates a more significant match, suggesting a true homologous relationship rather than a random similarity. Researchers use this value to filter results and identify novel alleles with high confidence based on their evolutionary.

**223.** (B) Identifying complex consensus motifs and domain profiles characteristic of divergent protein families
Hidden Markov Models uniquely solve the challenge of identifying complex consensus motifs and domain profiles characteristic of divergent protein families. By capturing the statistical probability of every amino acid at each position, HMMs can detect remote homologs that simple pairwise alignments might miss. This capability is essential for gene mining, allowing for the functional annotation of sequences that have.

**224.** (A) Polyubiquitination (specifically linked through Lysine 48)
Polyubiquitination, specifically linked through Lysine 48, is the structural modification that marks a protein for degradation by the 26S proteasome. This chain of ubiquitin molecules is recognized by the regulatory subunit of the proteasome, which then unfolds the target protein and translocates it into the catalytic core for proteolysis. This mechanism is essential for controlling protein levels and.

Crick base pairing is permitted at the first position of the anticodon, which corresponds to the third position of the codon. This flexibility ensures that a single tRNA can accurately recognize multiple synonymous codons, reducing the total metabolic.

**261.** (B) The structural or catalytic domains essential for protein function
In the sequence alignment of two evolutionary divergent paralogs, the regions with the highest conservation typically represent essential structural or catalytic domains. These functional motifs are critical for the protein's biological activity and are therefore maintained by purifying selection. While non-essential regions accumulate mutations over time, the conservation of these specific domains provides clues about the shared ancestral.

**262.** (A) It fails to identify genes with no known counterparts in existing databases (orphan genes).
The most significant disadvantage of purely homology-based in silico gene discovery is its failure to identify orphan genes. These are sequences that lack any known counterparts in existing databases and therefore cannot be recognized through similarity searches. Relying only on homology limits the discovery to previously characterized gene families, potentially missing entirely novel biological functions unique to the organism.

**263.** (C) Conserved, short non-coding sequence blocks upstream of homologous genes across species (phylogenetic footprinting)
When identifying cis-regulatory elements in silico, researchers often look for conserved, short non-coding sequence blocks upstream of homologous genes. This approach, known as phylogenetic footprinting, assumes that essential regulatory motifs are conserved across species due to their functional importance. By comparing divergent genomes, these shared blocks can be pinpointed as potential binding sites for transcription factors that control gene.

**264.** (A) In silico predictions cannot definitively confirm actual expression, post-transcriptional processing, or physiological functionality.
A fundamental limitation of in silico gene models is that they cannot definitively confirm actual expression or physiological functionality. While computational algorithms can predict gene structures based on sequence patterns and homology, experimental validation is required to prove that the gene is transcribed, correctly spliced, and produces a functional protein. In silico results remain hypotheses until verified through laboratory.

**265.** (B) Telomeres
Telomeres are the specialized DNA sequences located at the physical ends of linear eukaryotic chromosomes. They consist of tandem repeats and associated proteins that protect the chromosome from degradation and fusion. By providing a structural cap, telomeres maintain genomic integrity and prevent the cell from recognizing the natural ends of the DNA as double-strand breaks that require repair.

**266.** (C) Short tandem repeat satellite sequences
In most eukaryotes, short tandem repeat satellite sequences constitute the primary repetitive component of telomeres. For example, the human telomere contains thousands of repeats of the sequence TTAGGG. These repetitive regions provide a substrate for telomerase and form a protective loop structure known as the T-loop, which shields the chromosomal ends from enzymatic digestion and unwanted recombination events.

**267.** (B) DNA polymerase requires a primer and synthesizes exclusively $5'$ to $3'$, leaving a gap at the $5'$ end of the newly synthesized strand when the terminal RNA primer is removed.
DNA polymerase requires a primer and synthesizes exclusively $5'$ to $3'$, leaving a gap at the $5'$ end of the newly synthesized strand when the terminal RNA primer is removed. Consequently, the chromosomes shorten with each round.

**268.** (A) A reverse transcriptase carrying its own RNA template
Telomerase solves the end-replication problem by acting as a reverse transcriptase that carries its own RNA template. It uses this internal RNA sequence to extend the $3'$ end of the telomeric DNA through the addition of repeats. This elongation provides the necessary length for primase to synthesize a new primer, allowing the cell to maintain stable chromosome lengths.

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**234.** (A) A $5' - \text{to} - 3'$ exonuclease (like Xrn2) that degrades the downstream RNA attached to the polymerase
In the torpedo model of eukaryotic transcription termination, the role of the "torpedo" is played by a $5' - \text{to} - 3'$ exonuclease, such as Xrn2. After the nascent RNA is cleaved at the polyadenylation site, the exonuclease attaches to the new $5'$ end of the downstream fragment. It rapidly degrades the RNA and, upon reaching the polymerase, triggers its dissociation.

**235.** (A) II, IV, I, III
The operational sequence of a yeast two-hybrid assay begins with cloning gene $X$ in-frame with a DNA-binding domain and gene $Y$ in-frame with an activation domain. Yeast cells are then co-transformed with both plasmids to express the fusion proteins. Finally, the interaction is assessed by monitoring yeast growth on selective media lacking histidine, which indicates the activation of the.

**236.** (A) They permit the rapid isolation of corresponding genes from unsequenced or non-model species using degenerate primers.
A significant advantage of homology-based gene cloning is the ability to rapidly isolate corresponding genes from unsequenced or non-model species using degenerate primers. By targeting conserved sequences, researchers can bypass the need for prior genomic information, facilitating the study of evolutionary relationships and the identification of important functional genes in a wide range of diverse organisms across the.

**237.** (A) Steroid hormone receptors
Steroid hormone receptors are structurally notable for containing a zinc finger domain and commonly binding as homodimers to palindromic sequences. These transcription factors are activated by the binding of specific hormones, which induces their translocation into the nucleus. Once there, they recognize response elements in the promoters of target genes, regulating a wide variety of physiological and developmental.

**238.** (A) It catalyzes transesterification reactions utilizing precisely positioned hydroxyl groups and metal ions.
The fundamental catalytic mechanism of a ribozyme during RNA splicing involves transesterification reactions that utilize precisely positioned hydroxyl groups and metal ions. The RNA structure provides the active site necessary to facilitate the attack of a nucleophile on a phosphodiester bond. This process allows for the efficient excision of introns and ligation of exons without the requirement for.

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**239.** (B) A reporter gene assay showing decreased expression when the element is cloned upstream of an active promoter
A reporter gene assay provides the most direct evidence of silencer activity. In this experiment, the suspected element is cloned upstream of an active promoter driving a reporter gene. A significant decrease in the expression of the reporter compared to a control indicates that the element possesses the ability to repress transcription, confirming its functional identity as a.

**240.** (B) The spatial proximity of the $5'$ splice site and the branch point adenine for the first catalytic step
The interaction between snRNPs $U_1$ and $U_2$ during spliceosome assembly ensures the spatial proximity of the $5'$ splice site and the branch point adenine. By bringing these two distant regions together, the machinery facilitates the first catalytic transesterification step. This coordination is essential for the accurate removal of introns and the correct alignment of exons for subsequent ligation.

**241.** (A) The code is read continuously as triplets without any internal commas or spaces.
The genetic code is read continuously as triplets without any internal commas or spaces, meaning it lacks punctuation within the coding sequence. Once translation initiates at the start codon, the ribosome moves exactly three nucleotides at a time until it reaches a stop codon. This contiguous reading ensures that the amino acid sequence is produced accurately without interruption.

**242.** (A) The specific open reading frame to be translated
The universal start codon AUG strictly establishes the specific open reading frame to be translated. By defining the initiation point, it determines which set of nucleotide triplets will be read by the ribosome as codons. Any shift in the starting position would result in an entirely

**190.** (B) Because a single specific codon never specifies more than one amino acid.
The genetic code is described as unambiguous because a single specific codon never specifies more than one amino acid. While the code is degenerate, meaning multiple codons can code for the same amino acid, each individual codon has a unique and fixed assignment. this ensures that the genetic information is translated into a precise and consistent polypeptide sequence every.

**191.** (D) A single base pair insertion or deletion
A single base pair insertion or deletion is the specific type of mutation that fundamentally alters the reading frame of a translated mRNA. Because the genetic code is read in non-overlapping triplets, adding or removing a nucleotide shifts the entire sequence downstream. This "frameshift" mutation typically results in an entirely different amino acid sequence and often introduces.

**192.** (A) Aminoacyl-tRNA synthetases
Aminoacyl-tRNA synthetases are the cellular components that strictly enforce the accurate translation of the genetic code before the ribosome's involvement. These enzymes are responsible for covalently linking the correct amino acid to its corresponding tRNA. By ensuring high fidelity during this "charging" step, they establish the fundamental accuracy of protein synthesis, as the ribosome simply follows the instructions.

**193.** (C) An aminoacyl-tRNA, causing premature chain termination
The antibiotic puromycin inhibits translation by acting as a structural analog of an aminoacyl-tRNA. It enters the ribosomal A site and participates in peptide bond formation, which causes the nascent polypeptide chain to be released prematurely. Because puromycin lacks the rest of the tRNA structure, it cannot be translocated, effectively terminating protein synthesis and preventing the production of functional.

**194.** (A) The leucine zipper
The leucine zipper is a structural domain that allows transcription factors to form dimeric complexes prior to DNA binding. It consists of two alpha-helices with leucine residues that interdigitate to create a stable hydrophobic interface. This dimerization is essential for many regulatory proteins to achieve the correct conformation and affinity required to recognize and bind their specific target DNA sequences.

**195.** (A) II, IV, III, I
The assembly of the eukaryotic pre-initiation complex follows a sequential order. First, TFIID binds the promoter, followed by the recruitment of TFIIA and TFIIB. Next, RNA polymerase II enters the complex along with TFIIF. Finally, TFIIE and TFIIH join to complete the assembly. This coordinated process ensures that the transcription machinery is correctly positioned and ready to.

**196.** (A) Promoter escape and initiation of 5′ capping
The phosphorylation of the RNA polymerase II C-terminal domain at Serine 5 is primarily associated with promoter escape and the initiation of 5′ capping. This modification occurs shortly after transcription starts and recruits the enzymes needed to add the 7-methylguanosine cap to the nascent transcript. It signals the transition from the initiation phase to the early stages of elongation.

**197.** (D) DNase I footprinting
DNase I footprinting is the most appropriate technique to identify the specific nucleotide sequence where an unknown transcription factor binds a promoter. By protecting the DNA from enzymatic cleavage, the bound protein leaves a "footprint" that reveals its exact binding site. This method allows researchers to map the regulatory regions of a gene and understand how specific factors control.

**198.** (A) The transcriptional activation of a reporter gene (e.g., LacZ or $HIS_3$)
In the yeast two-hybrid system, a positive protein-protein interaction is visually or selectively identified by the transcriptional activation of a reporter gene. Common reporters include LacZ, which produces a blue color, or $HIS_3$, which allows yeast to grow on media lacking histidine. This activation confirms that the "bait" and "prey" proteins have successfully brought the transcription factor domains together.

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face of the cell membrane. These hydrophobic groups insert into the lipid bilayer, facilitating the localization of signaling proteins and other enzymes to their site of action. This modification is essential for proper cellular communication.

**252.** (A) Identifying naturally occurring alleles that confer resistance to stress or disease for crop improvement
The fundamental goal of gene mining in applied agricultural biotechnology is identifying naturally occurring alleles that confer resistance to stress or disease for crop improvement. By screening diverse germplasm or wild relatives, researchers can discover valuable genetic variants that can be introduced into elite varieties. This approach enhances the resilience and productivity of crops, contributing to global food.

**253.** (A) A short internal sequence rich in basic amino acids (lysine and arginine) known as an NLS
The sequence motif that directs a newly translated protein to be imported into the nucleus is known as a Nuclear Localization Signal. It typically consists of a short internal sequence rich in basic amino acids like lysine and arginine. This signal is recognized by importin proteins, which facilitate the transport of the polypeptide through the nuclear pore complex into.

**254.** (A) The final end product acts as an allosteric inhibitor of an early enzyme in the pathway.
Feedback inhibition in a biosynthetic pathway works when the final end product acts as an allosteric inhibitor of an early enzyme. By binding to a specific site on the enzyme, the product induces a conformational change that reduces its catalytic activity. This mechanism allows the cell to rapidly downregulate production when the end product reaches sufficient concentrations, preventing.

**255.** (A) By increasing the functional complexity of the proteome without requiring additional genes
Alternative splicing bypasses the constraints of genome size by increasing the functional complexity of the proteome without requiring additional genes. By producing multiple protein isoforms from a single genetic locus, organisms can expand their repertoire of biological functions. This efficiency allows complex eukaryotes to achieve sophisticated regulation and diverse phenotypes even with a relatively stable number of structural coding.

**256.** (A) A sharp boundary of histone modifications is observed, preventing heterochromatin from spreading into an active gene.
A sharp boundary of histone modifications provides strong experimental support for the existence of an insulator element. Insulators can act as barriers that prevent the spreading of repressive heterochromatin into adjacent active gene regions. By maintaining distinct epigenetic states on either side of the element, the insulator ensures that gene expression patterns are localized and not inappropriately silenced by.

**257.** (B) Histone deacetylase (HDAC)
An enzyme that specifically removes an acetyl group from the lysine residues of a histone tail is classified as a histone deacetylase. These enzymes reverse the action of histone acetyltransferases, leading to increased positive charge on the histones and a more condensed chromatin structure. HDAC activity is generally associated with transcriptional silencing and the maintenance of a repressive chromatin.

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**258.** (A) Transcriptional silencing and chromatin condensation
The most common phenotypic outcome of recruiting an HDAC complex to a promoter region is transcriptional silencing and chromatin condensation. By removing acetyl groups from histones, these enzymes promote a tighter interaction between the proteins and DNA. This reduces the accessibility of the promoter to the transcription machinery, effectively shutting down gene expression and contributing to stable epigenetic repression.

**259.** (A) Processing by Dicer and targeting by the RNAi machinery
When an RNA virus produces double-stranded RNA during its life cycle, the host defense mechanism will target it through the RNAi machinery. The enzyme Dicer will process the viral dsRNA into small fragments, which are then loaded into the RISC complex. These small RNAs guide the complex to cleave viral transcripts, effectively inhibiting viral replication and protecting the.

**260.** (A) Both A and R are true and R is the correct explanation of A.
The wobble hypothesis allows the genetic code to be translated efficiently by fewer than sixty-one tRNAs. This is possible because non-Watson-

**225.** (B) I, II, IV and V
Eukaryotic translation initiation involves a series of coordinated steps. eIF4E binds the $5'$ cap, and the pre-initiation complex scans the mRNA for the Kozak sequence. After the start codon is recognized, the large 60S subunit joins the complex, a process that requires GTP hydrolysis. All these statements accurately describe the initiation pathway, except for the specific tRNA.

**226.** (D) By causing the scanning ribosome to translate a short peptide and frequently dissociate before reaching the main start codon
Upstream open reading frames regulate the translation of the main downstream reading frame by causing the scanning ribosome to translate a short peptide and then frequently dissociate. Because the ribosome often fails to re-initiate at the primary start codon after finishing the uORF, the total amount of the main protein produced is significantly reduced. This mechanism provides precise control.

**227.** (A) Cellular stress such as amino acid starvation, viral infection, or ER stress
Cellular stress, such as amino acid starvation, viral infection, or endoplasmic reticulum stress, most potently activates the kinase that phosphorylates $eIF_2$ alpha. This modification is a key component of the integrated stress response, leading to a global shutdown of translation initiation. By reducing protein synthesis, the cell can conserve energy and resources to prioritize the repair and survival.

**228.** (B) It helps the innate immune system distinguish self mRNA from viral RNA.
The $2' - O$-methylation of the $5'$ cap structure in higher eukaryotes helps the innate immune system distinguish self mRNA from viral RNA. Many viruses produce transcripts that lack this specific modification, which can then be recognized by cellular sensors to trigger an antiviral response. By marking endogenous transcripts as "self," this modification prevents the activation of immune pathways against the.

**229.** (B) They inadvertently added a strong denaturant (like SDS) to the gel.
A likely technical reason for failing to see a shifted band in EMSA is the inadvertent addition of a strong denaturant like SDS. Because EMSA relies on the native interaction between proteins and DNA, any reagent that disrupts protein folding or binding will prevent the formation of the complex. Maintaining non-denaturing conditions throughout the incubation and electrophoresis is essential.

**230.** (C) Active gene promoters
In a ChIP analysis, an antibody against $H_3K_4me_3$ will primarily enrich genomic regions corresponding to active gene promoters. Trimethylation of Histone $H_3$ at Lysine 4 is a well-established epigenetic mark associated with transcriptionally active states. By mapping these locations, researchers can identify which genes are currently being expressed or are poised for activation in a given cell type.

**231.** (B) Between adjacent nucleotide pairs within a single gene
The concept of the recon, proposed by Benzer, implies that recombination can occur between adjacent nucleotide pairs within a single gene. This discovery showed that recombination is not limited to whole genes or large segments of chromosomes. Instead, the genetic material is divisible at the level of individual base pairs, allowing for precise rearrangements and high levels of variation.

**232.** (A) A specific nucleotide mutation simultaneously alters the amino acid sequence of two distinct viral proteins.
The definitive proof that two genes are overlapping is that a specific nucleotide mutation simultaneously alters the amino acid sequence of two distinct proteins. This occurs because the genes share the same DNA segment but use different reading frames. This dual impact of a single mutation highlights the extreme economy of viral genomes and the tight evolutionary constraints on.

**233.** (B) It lacks a precise termination site and stops downstream of the poly-A cleavage signal, followed by exonuclease action (torpedo model).
Eukaryotic RNA polymerase II lacks a precise termination site and typically stops downstream of the poly-A cleavage signal. Termination is followed by the action of a $5' - \text{to} - 3'$ exonuclease that degrades the remaining RNA attached to the enzyme, according to the torpedo model. This process ensures that the polymerase is effectively removed from the DNA once the mature transcript.

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**269.** (A) Unwinding the DNA at the promoter (helicase) and phosphorylating the RNA polymerase II CTD (kinase)
The activity of the general transcription factor TFIIH is crucial for the initiation and early elongation of transcription. It functions as a helicase to unwind the DNA at the promoter and as a kinase to phosphorylate the C-terminal domain of RNA polymerase II. These enzymatic steps allow the polymerase to escape the promoter and begin synthesizing the RNA transcript.

**270.** (A) Producing a non-functional mutant protein that blocks the activity of the normal wild-type protein, often by forming an inactive dimer
A dominant negative mutation in a transcription factor typically operates by producing a non-functional mutant protein that blocks the activity of the wild-type protein. Often, the mutant protein can still dimerize with the normal factor but creates an inactive complex that cannot bind DNA or activate transcription. This result interferes with the normal regulatory function, leading to a.

**271.** (B) The stringent response mediated by (p) ppGpp, downregulating all rRNA synthesis during amino acid starvation
The stringent response mediated by (p)ppGpp exemplifies a global regulatory mechanism in bacterial cells. In response to amino acid starvation, the concentration of this signaling molecule rises, leading to the downregulation of all ribosomal RNA synthesis. This coordinated response allows the cell to adjust its total translational capacity and metabolism across the entire genome, rather than just controlling a.

**272.** (B) Prevent the accumulation of potentially toxic truncated proteins resulting from premature stop codons
A key physiological function of nonsense-mediated decay is to prevent the accumulation of potentially toxic truncated proteins. By identifying and degrading mRNAs that contain premature stop codons, this pathway ensures that only full-length, functional polypeptides are synthesized. This surveillance mechanism protects the cell from the harmful effects of mutations and errors that occur during transcription or RNA processing events.

**273.** (B) By the cooperative binding of SR proteins to exonic splicing enhancers (ESEs) that recruit spliceosomal components to the authentic boundaries
The spliceosome distinguishes true splice sites from cryptic ones through the cooperative binding of SR proteins to exonic splicing enhancers. These proteins recruit essential spliceosomal components to the authentic intron-exon boundaries, ensuring accurate removal of non-coding sequences. This multifaceted recognition system relies on both the consensus sequences at the splice sites and additional regulatory motifs within the surrounding.

**274.** (A) It features a unique $2' - 5'$ phosphodiester bond connecting the $5'$ end of the intron to the branch point adenosine.
The RNA lariat intermediate is characterized by a unique $2' - 5'$ phosphodiester bond that connects the $5'$ end of the intron to the branch point adenosine. This structure is formed during the first catalytic step of splicing. The resulting circular loop with a linear tail contains the entire intron sequence and is subsequently released and degraded once the.

**275.** (B) An inability of RNA polymerase to bind and initiate transcription, resulting in zero expression
An operon system engineered to lack a promoter sequence will result in zero expression of all its genes. The promoter is the essential binding site for RNA polymerase, and without it, the enzyme cannot initiate transcription. Consequently, no messenger RNA will be synthesized, and the downstream structural genes will remain inactive, regardless of the presence of any other.

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**276.** (A) The $5'$ most base of the anticodon
The wobble base pairing between the codon UCU and its appropriate anticodon involves the first position at the $5'$ end of the anticodon. This flexibility allows the tRNA to interact with the third nucleotide of the codon, even if the pairing is non-standard. This mechanism is essential for the efficient translation of synonymous codons that specify the same.

**277.** (A) The halF-life of the transcript and its efficiency of translation
The presence of a poly-A tail on a eukaryotic mRNA significantly affects the transcript's halF-life and its translational efficiency. The tail protects the $3'$ end from exonucleases and facilitates the circularization of the mRNA through interactions with initiation factors.

These roles ensure that the transcript remains stable in the cytoplasm and is effectively recognized by the ribosomal machinery.

**278.** (A) It provides a near-universal dictionary translating 64 nucleotide triplets into 20 amino acids and 3 stop signals.
The universal genetic code provides a near-universal dictionary that translates sixty-four nucleotide triplets into twenty amino acids and three stop signals. It is characterized by degeneracy, meaning multiple codons can code for the same amino acid, but remains unambiguous as each codon specifies only one. This stability ensures the accurate and consistent translation of genetic information across almost all.

**279.** (B) Assess the kinetic complexity, reflecting the total length of unique sequences in the genome
The fundamental purpose of analyzing the $Cot_{\frac{1}{2}}$ value is to assess the kinetic complexity of a DNA sample. This value reflects the total length of unique sequences in the genome and provides a measure of its overall structural complexity. By determining how fast the DNA renatures, researchers can estimate the genome size and the proportion of repetitive sequences.

**280.** (A) Negative transcriptional control
A repressor binding to an operator strictly exemplifies negative transcriptional control. This regulatory mechanism works by physically blocking RNA polymerase from initiating transcription, thereby shutting down gene expression. This is a common strategy in prokaryotes, such as in the Lac and Trp operons, where the cell must respond quickly to changes in the availability of specific metabolic substrates.

**281.** (A) eIF4G
eIF4G is the scaffold protein that mediates the bridging interaction between the 5′ cap and the 3′ poly-A tail of eukaryotic mRNA. By binding to both eIF4E and the poly-A binding protein, eIF4G facilitates the circularization of the transcript. This loop structure enhances the efficiency of translation initiation and promotes the recycling of ribosomes for subsequent rounds.

**282.** (B) Acting as a competitive inhibitor of the $eIF_2B$ guanine nucleotide exchange factor, trapping $eIF_2$ in an inactive GDP-bound state
Phosphorylation of $eIF_2$ alpha prevents translation initiation by acting as a competitive inhibitor of $eIF_2B$, the guanine nucleotide exchange factor. This modification traps $eIF_2$ in an inactive GDP-bound state, preventing it from being recharged with GTP. Consequently, the factor cannot deliver the initiator tRNA to the ribosome, leading to a global shutdown of protein synthesis under stress conditions.

**283.** (A) Can stimulate transcription from a basal promoter irrespective of its orientation or considerable distance
An enhancer is defined by its ability to stimulate transcription from a basal promoter irrespective of its orientation or considerable distance. This cis-regulatory element can be located thousands of base pairs away, either upstream or downstream, and still significantly increase the gene's expression. This flexibility distinguishes enhancers from core promoter elements, which have fixed positions and orientations relative to.

**284.** (B) The DNA contains distinct classes of sequences differing widely in their repetition frequencies.
A multiphasic Cot curve indicates that the DNA sample contains distinct classes of sequences that differ widely in their repetition frequencies. Each phase of the curve represents the reassociation of a different fraction, such as highly repetitive, moderately repetitive, or unique DNA. This complex renaturation profile is characteristic of eukaryotic genomes, reflecting their elaborate structural organization and diverse genetic.

**285.** (B) Recruit chromatin-remodeling complexes that establish a repressive heterochromatin state
A protein that specifically recognizes heavily methylated CpG dinucleotides most likely functions to recruit chromatin-remodeling complexes. These complexes then establish a repressive heterochromatin state, leading to long-term gene silencing. By binding to methylated DNA, these proteins serve as a bridge between epigenetic marks and the machinery that physically condenses chromatin, ensuring the stable suppression of specific genomic regions.

**286.** (A) Acting as an RNA molecule with intrinsic catalytic enzymatic activity
A ribozyme is characterized by its capability to act as an RNA molecule with intrinsic catalytic enzymatic activity. These specialized RNAs can perform various biochemical reactions, such as peptide bond formation in the ribosome or the

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excision of introns during splicing. The discovery of ribozymes highlighted the functional versatility of RNA, showing it can store information and catalyze essential.

**287.** (A) ER-associated degradation (ERAD) involving retrotranslocation and ubiquitination
ER-associated degradation is the process that ensures improperly folded proteins in the endoplasmic reticulum are not accumulated. It involves the identification of misfolded polypeptides, their retrotranslocation into the cytosol, and subsequent ubiquitination. These tagged proteins are then degraded by the 26S proteasome. This quality control mechanism is vital for maintaining cellular protein homeostasis and preventing the toxicity associated.

**288.** (A) A microRNA (miRNA)
A short RNA of approximately twenty-two nucleotides that exhibits imperfect complementarity to the 3' UTR of target genes is almost certainly a microRNA. These endogenous molecules regulate gene expression by guiding the RISC complex to repress translation or promote mRNA decay. Their imperfect pairing allows a single miRNA to target multiple different transcripts, coordinating complex gene regulatory networks.

**289.** (A) Classical genes were assumed to be continuous linear units of information, while modern molecular genes may be discontinuous sequences separated by introns.
Classical Mendelian concepts viewed the gene as a continuous linear unit of information, whereas modern molecular definitions acknowledge that genes may be discontinuous. The discovery of split genes revealed that eukaryotic coding sequences are often separated by non-coding introns that are removed during splicing. This transition reflects a more complex understanding of how genetic information is stored and processed.

**290.** (A) It monitors the geometry of the codon-anticodon pairing in the minor groove of the A site.
The 16S rRNA ensures translational fidelity by monitoring the geometry of the codon-anticodon pairing in the ribosomal A site. It forms specific hydrogen bonds with the minor groove of the correctly matched helix, inducing a conformational change that triggers the next step of translation. This precise sensing mechanism allows the ribosome to discriminate against incorrect tRNAs with high accuracy.

**291.** (A) Higher eukaryotes possess substantially more introns per gene and complex regulatory splicing factors allowing diverse isoform generation.
Alternative splicing plays a significantly larger role in higher eukaryotes because they possess substantially more introns per gene and a greater variety of complex regulatory splicing factors. These features allow for the generation of many diverse protein isoforms from a single gene. This expanded capacity for proteomic diversity is a key factor in the phenotypic complexity and specialized cell.

**292.** (A) $E_3$ (Ubiquitin ligase)
In the ubiquitin-proteasome pathway, the $E_3$ ubiquitin ligase is the enzyme that specifically dictates substrate recognition and specificity. It serves as the bridge that brings the target protein and the ubiquitin-conjugating enzyme together. By identifying specific degradation signals, or degrons, on the substrate, the $E_3$ ligase ensures that only the appropriate proteins are tagged for destruction by the proteasome.

**293.** (A) Aligning full-length cDNA or RNA-seq data against the genomic sequence
Aligning full-length cDNA or RNA-seq data against the genomic sequence provides the most robust bioinformatic validation of a predicted gene model. This comparison provides experimental evidence of the actual transcript structure, including the exact locations of exons and splice sites. By confirming that the predicted gene is actually transcribed and processed, researchers can verify the accuracy of computational.

**294.** (A) A distinct, recurrent 3D structural folding pattern that typically mediates specific DNA binding or dimerization
A motif in the context of transcription factors is strictly defined as a distinct, recurrent three-dimensional structural folding pattern. These patterns, such as the zinc finger or leucine zipper, typically mediate specific DNA binding or dimerization. Understanding these motifs allows researchers to predict the DNA-binding properties and regulatory roles of newly discovered proteins based on their characteristic secondary and tertiary.

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**295.** (A) Intrinsic termination relies on the specific formation of an RNA hairpin followed by a weak poly-U tract, while rho-dependent lacks the poly-U requirement and requires a rut site.
Intrinsic termination relies on the specific formation of an RNA hairpin followed by a weak poly-U tract, which causes the transcription complex to dissociate. In contrast, rho-dependent termination lacks the poly-U requirement and necessitates the binding of the rho protein to a rut site on the transcript. Structural differences in the emerging RNA and the DNA template dictate.

**296.** (A) Transcription occurs in the nucleus, followed by mRNA export, and subsequent translation in the cytoplasm.
In a typical eukaryotic cell, transcription occurs in the nucleus, followed by mRNA export and subsequent translation in the cytoplasm. This spatial and temporal separation prevents the two processes from occurring simultaneously. The compartmentalization allows for extensive post-transcriptional processing and provides multiple levels of regulation that are not available in prokaryotes, where transcription and translation are tightly coupled in.

**297.** (A) The tight spatial and temporal coupling of RNA polymerase advancing on DNA and the ribosome advancing on the nascent RNA transcript
Transcriptional attenuation relies entirely on the tight spatial and temporal coupling of RNA polymerase advancing on DNA and the ribosome advancing on the nascent RNA. In prokaryotes, this proximity allows the speed of the ribosome to directly influence the secondary structure of the transcript as it is being synthesized. This coupling is the physical basis for sensing metabolic levels.

**298.** (A) A guanylyltransferase adding a GMP in a $5' - 5'$ linkage followed by methylation
The capping of eukaryotic pre-mRNA is characterized by a guanylyltransferase adding a GMP molecule in a unique $5' - 5'$ linkage. This is followed by the methylation of the guanine at the $N_7$ position. This enzymatic process occurs cotranscriptionally and provides the 7-methylguanosine cap that protects the $5'$ end of the transcript from degradation and facilitates subsequent steps in.

**299.** (A) Transcript cleavage or the severe repression of successful translation
The binding of the RISC complex to a target mRNA most frequently leads to transcript cleavage or the severe repression of successful translation. Depending on the complementarity of the guide RNA, the Argonaute protein can either cut the transcript directly or block the recruitment of the ribosome. Both outcomes effectively silence the gene by preventing the production of a.

**300.** (A) Statistically score the probability of a sequence belonging to a specific conserved protein family using complex position-specific profiles
Hidden Markov Models are highly favored in modern gene mining algorithms due to their ability to statistically score the probability of a sequence belonging to a specific conserved protein family. By using complex position-specific profiles, HMMs can identify essential functional domains and motifs even in highly divergent sequences. This probabilistic approach is far more sensitive than simple similarity.

**********

NODIA

# CHAPTER 04

# Genetic Engineering and Recombinant DNA Technology

2007

**1.** Which wavelength is primarily used to monitor the purity of DNA preparations by detecting protein contamination?
(A) 230 nm
(B) 260 nm
(C) 280 nm
(D) 320 nm

2023

**2.** The hyperchromic effect observed during DNA denaturation is due to which molecular event?
(A) Disruption of phosphodiester bonds
(B) Cleavage of hydrogen bonds with water
(C) Unstacking of nitrogenous bases
(D) Methylation of cytosine residues

2021

**3.** What describes the sequence complexity factor in DNA renaturation kinetics (Cot curve)?
(A) Total length of unique DNA sequences
(B) Specific rate at which DNA degrades
(C) Number of repeating structural genes
(D) Total amount of uracil incorporated

2014

**4.** Which parameter is directly proportional to the melting temperature ($T_m$) of double-stranded DNA?
(A) Adenine-Thymine content
(B) Uracil-Adenine content
(C) Length of the poly-A tail
(D) Guanine-Cytosine content

2021

**5.** In a standard phenol-chloroform DNA extraction, which component remains in the upper aqueous phase?
(A) Denatured proteins
(B) Nucleic acids
(C) Lipids
(D) Carbohydrates

2008

**6.** What is the primary function of adding isoamyl alcohol during organic DNA extraction?
(A) To precipitate the DNA
(B) To dissolve the nuclear membrane
(C) To prevent foaming
(D) To neutralize the pH

2016

**7.** Which chemical is routinely used for the precipitation of purified DNA from an aqueous solution?
(A) Phenol
(B) Chloroform
(C) Sodium dodecyl sulfate
(D) Isopropanol

2025

**8.** Identify the correct role of EDTA in nucleic acid extraction buffers.
(A) It acts as a chelating agent for divalent cations
(B) It functions to stabilize the RNA polymerase
(C) It serves to denature various cellular proteins
(D) It buffers the solution pH to a value of 8.0

2001

**9.** Why is guanidinium thiocyanate frequently used in RNA isolation protocols?
(A) It specifically binds to mRNA poly-A tail sequences
(B) It acts as a strong RNase inhibitor and chaotropic agent
(C) It facilitates the selective degradation of genomic DNA
(D) It provides the optimal pH levels for RNA stability

2019

**10.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Plasmids can be separated from genomic DNA using alkaline lysis.
**Reason (R):** Linear genomic DNA denatures and aggregates at high pH, whereas covalently closed circular plasmid DNA strands remains physically linked.

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(B) Ampicillin resistance gene
(C) Centromeric sequence (CEN)
(D) Multiple cloning site

2021
**53.** A strong inducible promoter is generally preferred over a strong constitutive promoter in expression vectors to prevent:
(A) Rapid plasmid degradation
(B) Toxicity to the host cell during early growth
(C) Immediate secretion of the protein
(D) Transcriptional termination failure

2021
**54.** The Shine-Dalgarno sequence in a prokaryotic expression vector functions as a:
(A) Transcriptional terminator
(B) Ribosome binding site for translation initiation
(C) Promoter binding site for RNA polymerase
(D) Restriction enzyme recognition site

2010
**55.** Determine the correct order of functional elements from 5' to 3' in a standard prokaryotic expression vector.
(A) Ribosome binding site → Promoter → Start codon → Multiple cloning site → Terminator
(B) Promoter → Ribosome binding site → Start codon → Multiple cloning site → Terminator
(C) Promoter → Multiple cloning site → Ribosome binding site → Start codon → Terminator
(D) Multiple cloning site → Promoter → Ribosome binding site → Start codon → Terminator

2016
**56.** Which purification tag utilizes immobilized metal affinity chromatography (IMAC) targeting nickel or cobalt ions?
(A) GST tag
(B) FLAG tag
(C) Polyhistidine (His) tag
(D) MBP tag

2019
**57.** Synthetic double-stranded oligonucleotides containing one or more restriction sites that are blunt-end ligated to DNA fragments are called:
(A) Adaptors
(B) Linkers
(C) Promoters
(D) Terminators

2003
**58.** Adaptors differ from linkers primarily because adaptors:
(A) Are always single-stranded
(B) Already possess preformed cohesive (sticky) ends
(C) Require restriction enzyme digestion before ligation
(D) Cannot be synthesized chemically

2004
**59.** In cDNA library construction, homopolymer tailing involves adding a string of identical nucleotides to the 3' ends using:
(A) DNA polymerase I
(B) Terminal deoxynucleotidyl transferase
(C) $T_4$ DNA ligase
(D) Reverse transcriptase

2011
**60.** Match the Gene Delivery Method in List I with its underlying mechanism in List II.
List I: (Gene Delivery Method)
P. Electroporation
Q. Lipofection
R. Microinjection
S. Calcium phosphate
List II: (Mechanism)
1. Direct mechanical insertion using a fine needle
2. Co-precipitation of DNA to facilitate endocytosis
3. Application of brief electrical pulses to create transient pores
4. Encapsulation of DNA in artificial lipid vesicles
(A) P-3, Q-4, R-1, S-2
(B) P-4, Q-3, R-2, S-1
(C) P-3, Q-1, R-4, S-2
(D) P-1, Q-4, R-3, S-2

2013
**61.** Chemical competence in E. coli is routinely induced by treating the cells with:
(A) High concentrations of EDTA
(B) Ice-cold calcium chloride solution
(C) Sodium dodecyl sulfate
(D) Polyethylene glycol

2002
**62.** Which method guarantees the direct delivery of DNA into the nucleus of a target animal cell?
(A) Lipofection
(B) Calcium phosphate transfection
(C) Microinjection
(D) Electroporation

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P. Type I
Q. Type II
R. Type III
S. Type IV

List II: (Characteristic)

1. Cleaves specifically at methylated DNA
2. Cleaves at random sites up to 1000 bp away from recognition site
3. Needs no ATP, cleaves exactly at or near recognition site
4. Cleaves about 25 bp away from recognition site

(A) P-2, Q-3, R-4, S-1
(B) P-1, Q-4, R-3, S-2
(C) P-3, Q-1, R-2, S-4
(D) P-2, Q-4, R-1, S-3

2010

**21.** Restriction enzymes that recognize the same DNA sequence but cleave at different positions within that sequence are called:
(A) Isoschizomers
(B) Neoschizomers
(C) Isocaudomers
(D) Epimers

2024

**22.** BamHI recognizes the sequence $G_{GATCC}$. What type of ends does it generate upon cleavage?
(A) Blunt ends
(B) 3' protruding ends
(C) 5' protruding ends
(D) 5' blunt and 3' protruding ends

2024

**23.** Star activity in restriction endonucleases refers to:
(A) The enzyme's ability to act on RNA
(B) High-efficiency cleavage in optimal buffer
(C) The requirement of an activator protein
(D) Relaxation of sequence specificity under suboptimal conditions

2024

**24.** Which condition is least likely to induce star activity in a restriction enzyme digest?
(A) Inclusion of magnesium ions at recommended levels
(B) High glycerol concentration
(C) Non-optimal buffer pH
(D) High concentration of enzyme

2021

**25.** Determine the correct order of steps for resolving DNA fragments via agarose gel electrophoresis.
I. Applying electric field
II. Casting the agarose gel with a fluorescent dye
III. Loading DNA samples mixed with tracking dye
IV. Visualizing under UV light

Which of the following represents the correct sequence of steps?
(A) I, II, III, IV
(B) II, III, I, IV
(C) II, I, III, IV
(D) III, II, I, IV

2006

**26.** In PCR, the annealing temperature is typically set based on the:
(A) Melting temperature of the template DNA
(B) Melting temperature of the primers
(C) Optimal temperature for Taq polymerase
(D) Extension time required

2023

**27.** Polyacrylamide gel electrophoresis (PAGE) is superior to agarose gel electrophoresis for:
(A) Resolving small DNA fragments with single-base differences
(B) Resolving very large genomic DNA fragments
(C) Rapid preparation of the gel matrix
(D) Lower toxicity during preparation

2021

**28.** Which component in a standard PCR master mix provides the essential divalent cations for Taq polymerase activity?
(A) KCl
(B) Tris-HCl
(C) $MgCl_2$
(D) EDTA

2023

**29.** Ethidium bromide allows visualization of DNA in gels through which specific mechanism?
(A) Covalent bonding to the sugar-phosphate backbone
(B) Ionic interaction with terminal phosphates
(C) Hydrogen bonding to thymine residues
(D) Intercalation between stacked nitrogenous bases

2003

**30.** Consider the following statements regarding Reverse Transcriptase (RT):
I. It requires an RNA template to synthesize cDNA.
II. It possesses 3' to 5' proofreading exonuclease activity.
III. It uses a primer to initiate synthesis.

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(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2003

**11.** Which method is most appropriate for isolating highly pure mRNA from total eukaryotic RNA?
(A) Oligo(dT) cellulose affinity chromatography
(B) Sucrose density gradient fractionation centrifugation
(C) Anion exchange chromatography using specific resins
(D) Gel filtration chromatography for size exclusion

2007

**12.** During the alkaline lysis method of plasmid isolation, what is the specific role of potassium acetate?
(A) To facilitate the lysis of the bacterial cell wall
(B) To effectively maintain a strictly alkaline pH
(C) To selectively precipitate denatured genomic DNA and proteins
(D) To promote the supercoiling of the plasmid DNA

2017

**13.** Which DNA modifying enzyme requires ATP to catalyze the formation of a phosphodiester bond between adjacent 3'-OH and 5'-P termini?
(A) Taq DNA polymerase
(B) Alkaline phosphatase
(C) Polynucleotide kinase
(D) $T_4$ DNA ligase

2003

**14.** What is the precise function of alkaline phosphatase in molecular cloning?
(A) It adds phosphate groups to the 5' ends of DNA
(B) It methylates DNA to protect it from restriction
(C) It removes phosphate groups from the 5' ends to prevent selF-ligation
(D) It synthesizes RNA primers

2014

**15.** Choose the correct sequence of enzymatic actions required to radiolabel a 5' dephosphorylated DNA fragment using [gamma-32P] ATP.
(A) Treatment with alkaline phosphatase → Forward reaction with Polynucleotide Kinase → Purification
(B) Treatment with terminal transferase → Exchange reaction with Polynucleotide Kinase → Purification
(C) Treatment with RNase H → Forward reaction with Polynucleotide Kinase → Purification
(D) Exchange reaction with Polynucleotide Kinase → Treatment with alkaline phosphatase → Purification

2010

**16.** Terminal deoxynucleotidyl transferase (TdT) is unique among DNA polymerases because it:
(A) Requires an RNA template
(B) Possesses 3' to 5' exonuclease activity
(C) Can only add pyrimidines
(D) Does not require a template strand

2008

**17.** Which enzyme is most appropriate for generating blunt ends from DNA fragments with 5' overhangs?
(A) Klenow fragment
(B) $T_4$ DNA ligase
(C) $S_1$ nuclease
(D) RNase A

2017

**18.** Identify the incorrect statement regarding $T_4$ Polynucleotide Kinase (PNK).
(A) It catalyzes the transfer of the gamma-phosphate of ATP
(B) It requires a 5' hydroxyl terminus on the substrate
(C) It operates optimally in the absence of magnesium ions
(D) It can perform an exchange reaction on 5'-phosphorylated DNA

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2010

**19.** Type II restriction endonucleases are preferred in recombinant DNA technology primarily because they:
(A) Cleave DNA at specific sites within or adjacent to their recognition sequence
(B) Require ATP and S-adenosylmethionine for the cleavage process
(C) Cleave DNA at random sites far from the actual recognition sequence
(D) Methylate the target site prior to performing the cleavage

2004

**20.** Match the Restriction Enzyme Type in List I with its specific characteristic in List II.
List I: (Restriction Enzyme Type)

2014

**42.** BAC vectors are primarily based on the replicon of which naturally occurring element?
(A) 2-micron circle of yeast
(B) Lambda phage genome
(C) F$^-$plasmid of E. coli
(D) Ti plasmid of Agrobacterium

2017

**43.** A unique structural component required for the stability of a Yeast Artificial Chromosome (YAC) but not a BAC is:
(A) Telomeric sequences
(B) A selectable marker
(C) An origin of replication
(D) Multiple cloning site

2014

**44.** Mammalian Artificial Chromosomes (MACs) are advantageous over YACs for human gene therapy because MACs:
(A) Replicate much faster in bacteria
(B) Have a smaller carrying capacity
(C) Utilize viral capsids for delivery
(D) Avoid random integration and position effects

2006

**45.** Consider the following statements concerning retroviral vectors:
I. They integrate their genome into the host cell DNA.
II. They require actively dividing cells for nuclear entry (except lentiviruses).
III. They package single-stranded RNA.
IV. They frequently cause transient, non-integrating expression.
V. They utilize reverse transcriptase within the host cell.
Which of the above statements are correct?
(A) I, II, III and V
(B) I, III and V
(C) II, III, IV and V
(D) I, II and IV

2022

**46.** Which viral vector system relies on a large double-stranded DNA genome and is primarily used for transient expression without genomic integration?
(A) Retrovirus
(B) Lentivirus
(C) Adeno-associated virus (AAV)
(D) Adenovirus

2006

**47.** Vaccinia virus vectors are unique because their transcription and replication occur exclusively in the:
(A) Host cell cytoplasm
(B) Host cell nucleus
(C) Endoplasmic reticulum
(D) Extracellular space

2016

**48.** In the baculovirus expression vector system (BEVS), recombinant proteins are typically produced in which host cells?
(A) Spodoptera frugiperda ($Sf_9$) insect cells
(B) E. coli
(C) Saccharomyces cerevisiae
(D) Chinese hamster ovary (CHO) cells

2014

**49.** Shuttle vectors are defined by their ability to:
(A) Transport proteins across the plasma membrane
(B) Replicate in two different host species
(C) Convert RNA into DNA
(D) Silence specific target genes

2023

**50.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Pichia pastoris is often favored over Saccharomyces cerevisiae for recombinant protein expression.
**Reason (R):** Pichia pastoris has a strong, tightly regulated AOX1 promoter and secretes fewer endogenous proteins.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2025

**51.** The 2-micron circle is a naturally occurring high-copy plasmid found in:
(A) Escherichia coli
(B) Saccharomyces cerevisiae
(C) Agrobacterium tumefaciens
(D) Bacillus subtilis

2012

**52.** Which structural feature ensures that an autonomously replicating sequence (ARS) vector in yeast is stably segregated during mitosis?
(A) Telomeric sequence (TEL)

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Which of the above statement(s) is/are correct?
(A) I and II
(B) I and III
(C) II and III
(D) I, II and III

2002
**31.** What is the specific role of RNase H during second-strand cDNA synthesis?
(A) It degrades double-stranded RNA
(B) It degrades the RNA strand of an RNA-DNA hybrid
(C) It synthesizes RNA primers
(D) It ligates the Okazaki fragments

2018
**32.** Which molecular strategy is commonly utilized to ensure full-length cDNA synthesis specifically targeting the 5' ends of mRNA?
(A) Random hexamer priming
(B) Oligo(dT) priming
(C) Sanger sequencing
(D) RACE (Rapid Amplification of cDNA Ends)

2025
**33.** The avian myeloblastosis virus (AMV) reverse transcriptase is often chosen over Moloney murine leukemia virus (MMLV) RT because AMV RT:
(A) Possesses higher thermal stability
(B) Lacks RNase H activity entirely
(C) Synthesizes RNA from DNA templates
(D) Requires no primer

2006
**34.** In the classical hairpin method for double-stranded cDNA synthesis, which enzyme is required to cleave the hairpin loop?
(A) Restriction endonuclease
(B) RNase H
(C) $S_1$ nuclease
(D) DNA ligase

2017
**35.** Plasmid vectors must contain an origin of replication (ori) to ensure:
(A) Independent replication within the host cell
(B) Integration into the host chromosome
(C) Expression of the cloned gene
(D) Conjugation between bacterial cells

2015
**36.** Which feature of the pUC vector series allows for alpha-complementation screening?
(A) The entire beta-galactosidase gene
(B) The ampicillin resistance gene
(C) The $T_7$ promoter
(D) The lacZ alpha peptide coding sequence

2005
**37.** Bacteriophage lambda vectors are superior to standard plasmid vectors for generating genomic libraries because they:
(A) Do not require a host cell for replication
(B) Can accommodate significantly larger DNA inserts
(C) Replicate as linear DNA exclusively
(D) Automatically sequence the cloned inserts

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2010
**38.** Phagemids are engineered cloning vectors that uniquely combine the features of:
(A) Plasmids and Cosmids
(B) Lambda phages and BACs
(C) Plasmids and $M_{13}$ bacteriophages
(D) YACs and Cosmids

2018
**39.** $M_{13}$ bacteriophage vectors are particularly useful for producing:
(A) Double-stranded circular DNA for restriction mapping
(B) Large genomic libraries
(C) Recombinant proteins in mammalian cells
(D) Single-stranded DNA for sequencing and mutagenesis

2010
**40.** Consider the following statements regarding cosmids:
I. They contain the cos site of bacteriophage lambda.
II. They can be packaged in vitro into phage heads.
III. They replicate via the lytic cycle and lyse the host cell.
IV. They can carry inserts up to 45 kb.
Which of the above statements is/are incorrect?
(A) I and II
(B) II and III
(C) III
(D) III and IV

2012
**41.** Which vector type provides the largest insert capacity for complex genome projects?
(A) Cosmid
(B) BAC (Bacterial Artificial Chromosome)
(C) YAC (Yeast Artificial Chromosome)
(D) Plasmid

2009

**63.** Recombinant clones in a pBR322 vector exhibit sensitivity to tetracycline but resistance to ampicillin. This phenomenon is known as:
(A) Alpha-complementation
(B) Directed evolution
(C) Transfection
(D) Insertional inactivation

2024

**64.** In blue-white screening, what enzymatic activity results in the formation of blue colonies?
(A) Alkaline phosphatase
(B) Beta-lactamase
(C) Beta-galactosidase
(D) Luciferase

2001

**65.** Determine the correct order of steps for colony hybridization.
I. Autoradiography or detection
II. Hybridization with a labeled probe
III. Transfer of colonies to a nitrocellulose membrane
IV. Lysis of cells and denaturation of DNA
Which of the following represents the correct sequence of steps?
(A) III, IV, II, I
(B) III, II, IV, I
(C) IV, III, II, I
(D) II, III, IV, I

2016

**66.** Which chemical agent is commonly used for in vitro mutagenesis by causing transitions from C to U through deamination?
(A) Sodium bisulfite
(B) Ethidium bromide
(C) EMS (Ethyl methanesulfonate)
(D) UV radiation

2024

**67.** The QuikChange site-directed mutagenesis protocol utilizes which enzyme to specifically degrade the methylated, non-mutated parental DNA template?
(A) EcoRI
(B) DpnI
(C) Taq polymerase
(D) Exonuclease III

2002

**68.** Directed evolution differs from rational design because directed evolution:
(A) Relies on random mutagenesis and screening for desired traits
(B) Requires extensive prior knowledge of the protein's 3D structure
(C) Uses only specific primer extensions
(D) Occurs entirely in vivo without human intervention

2010

**69.** Cassette mutagenesis involves:
(A) Amplifying the entire vector via PCR
(B) Replacing a restriction fragment with a mutant segment
(C) Chemically modifying the entire genome
(D) Introducing transposons randomly

2024

**70.** Consider the following statements regarding reverse genetics:
I. It starts with a known phenotype to map the responsible gene.
II. It starts with a known gene sequence and seeks to determine its phenotype.
III. Gene knockout is a typical technique used in reverse genetics.
Which of the above statements are correct?
(A) I and II
(B) I and III
(C) II and III
(D) I, II and III

2002

**71.** In creating a knockout mouse model, homologous recombination is typically performed first in which cell type?
(A) Embryonic stem (ES) cells
(B) Mature oocytes
(C) Fibroblasts
(D) Hematopoietic stem cells

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2025

**72.** The Cre-loxP system is widely utilized in mammalian genetics to achieve:
(A) Random insertional mutagenesis
(B) Conditional or tissue-specific gene knockouts
(C) Increased translation rates
(D) Transcriptional silencing via RNAi

2015

**73.** Which functional genomic tool is best suited for assessing the simultaneous expression of thousands of genes?
(A) Northern blotting
(B) DNA microarrays
(C) Site-directed mutagenesis
(D) Southern blotting

(D) Is significantly larger in terms of total base pairs than the genomic library

2017

**116.** The pBR322 vector was rationally designed to include unique restriction sites within:
(A) The ampicillin and tetracycline resistance genes
(B) The origin of replication
(C) The lacZ gene
(D) The $T_7$ promoter

2012

**117.** Bacteriophage lambda packaging places strict constraints on the size of the DNA insert. What is the approximate functional size range for a lambda vector packaged in vitro?
(A) 5 - 10 kb
(B) 10 - 25 kb
(C) 38 - 52 kb
(D) 75 - 100 kb

2010

**118.** Which unique genetic element enables an $M_{13}$ vector to exist as a single-stranded circular DNA inside the phage particle?
(A) Origin of replication (f1 ori)
(B) cos site
(C) Centromere
(D) Long terminal repeats

2003

**119.** A key advantage of using yeast host-vector systems over bacterial systems for recombinant protein production is:
(A) Capacity for eukaryotic post-translational modifications like glycosylation
(B) Faster replication time
(C) Inability to form inclusion bodies
(D) Lower cost of media

2011

**120.** Consider the following statements regarding Yeast Artificial Chromosomes (YACs):
I. They can carry inserts larger than 1 Megabase.
II. They are highly stable and rarely suffer from chimerism.
III. They contain ARS, CEN, and TEL sequences.
IV. They are routinely propagated in E. coli without modification.
Which of the above statements is/are incorrect?
(A) II and IV
(B) I and III
(C) I and IV
(D) II and III

2023

**121.** Viral vectors derived from adenoviruses are frequently utilized for gene therapy because they:
(A) Can transduce both dividing and non-dividing cells efficiently
(B) Provide permanent, integrated gene expression
(C) Specifically target only dividing cells
(D) Package RNA instead of DNA

2004

**122.** The baculovirus polyhedrin promoter is highly valued in recombinant expression systems because it is:
(A) Extremely strong and active in the very late phase of the infection cycle
(B) Constitutively active during all stages of infection
(C) Active only in mammalian cells
(D) Easily repressed by the addition of tetracycline

2003

**123.** Transcriptional terminators are included in expression vectors primarily to:
(A) Prevent transcriptional read-through which could destabilize the plasmid
(B) Stop translation at the correct amino acid
(C) Enhance the binding of ribosomes
(D) Serve as restriction enzyme recognition sites

2025

**124.** When designing a protein expression vector, the fusion of a signal peptide sequence to the N-terminus of the target protein ensures:
(A) Secretion of the recombinant protein into the periplasm or culture medium
(B) Protection against intracellular proteases
(C) Immediate degradation of the protein
(D) Aggregation into inclusion bodies

2018

**125.** Consider the following statements regarding the use of adaptors in library construction:
I. They are double-stranded oligonucleotides.
II. One end is blunt and the other has a predefined cohesive overhang.
III. The cohesive end is usually dephosphorylated to prevent selF-ligation.
IV. They are always ligated using RNA ligase.
V. They are used to add restriction sites to blunt-ended DNA fragments.
Which of the above statements are correct?
(A) I, II, III and V

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chains separately in E. coli.
II. The separate chains are purified and then oxidized to form disulfide bonds.
III. It completely lacks the C-peptide found in proinsulin.
IV. It is universally produced only in mammalian cell culture.
V. It was the first recombinant therapeutic protein approved for human use.

Which of the above statements are correct?
(A) I, II, III and V
(B) II, III, IV and V
(C) I, III, IV and V
(D) I, II, IV and V

2013
**86.** Recombinant subunit vaccines offer which major advantage over traditional live-attenuated vaccines?
(A) They possess absolutely zero risk of reverting to virulence
(B) They require fewer booster doses
(C) They naturally elicit stronger cytotoxic T-cell responses
(D) They do not require any adjuvants

2007
**87.** Strain improvement in industrial microbial genetics traditionally relies on which process before the advent of recombinant DNA?
(A) Random mutagenesis followed by rigorous screening
(B) Metabolic flux analysis
(C) CRISPR-Cas9 editing
(D) Transcriptome profiling

2015
**88.** Metabolic engineering to maximize the yield of a specific metabolite often involves:
(A) Deleting competing metabolic pathways
(B) Inhibiting cell growth completely
(C) Using non-renewable carbon sources exclusively
(D) Reducing the copy number of rate-limiting enzymes

2006
**89.** Single Cell Protein (SCP) production primarily utilizes microorganisms to provide high levels of:
(A) Proteins for animal or human consumption
(B) Lipids
(C) Dietary fiber
(D) Complex carbohydrates

2010
**90.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Lipid nanoparticles (LNPs) are highly effective vectors for delivering mRNA vaccines.
**Reason (R):** Ionizable lipids in LNPs remain neutral at physiological pH but become protonated in endosomes to facilitate endosomal escape.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2017
**91.** PEGylation of polymeric nanocarriers is primarily performed to:
(A) Evade the reticuloendothelial system and increase circulation time
(B) Increase targeting to specific tumor cells
(C) Promote rapid renal clearance
(D) Enhance the degradation of the encapsulated drug

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2015
**92.** The enhanced permeability and retention (EPR) effect is a phenomenon that allows nanocarriers to passively target:
(A) The blood-brain barrier
(B) Healthy liver tissue
(C) Solid tumors
(D) Skeletal muscle

2022
**93.** In plant transgenesis, the Ti plasmid is naturally derived from which organism?
(A) Escherichia coli
(B) Agrobacterium tumefaciens
(C) Bacillus thuringiensis
(D) Saccharomyces cerevisiae

2001
**94.** The T-DNA region of the wild-type Ti plasmid is defined by:
(A) Multiple cloning sites
(B) The vir region
(C) Origin of replication
(D) 25 bp direct repeat border sequences

2008
**95.** Which gene is widely utilized as a scorable reporter gene in plant transgenesis studies?

2016

**74.** Dicer is classified enzymatically as an:
(A) RNase III-like endonuclease
(B) RNA-dependent RNA polymerase
(C) RNA ligase
(D) RNase H-like exonuclease

2007

**75.** The key catalytic component within the RNA-induced silencing complex (RISC) responsible for target mRNA cleavage is:
(A) Drosha
(B) Exportin-5
(C) Argonaute (Ago)
(D) TRBP

2001

**76.** Unlike siRNAs, endogenous microRNAs (miRNAs) predominantly silence genes through:
(A) Imperfect base pairing leading to translational repression
(B) Perfect base pairing leading to mRNA cleavage
(C) Direct binding to the DNA promoter
(D) Degrading the ribosomal subunits

2016

**77.** Which molecule serves as the precursor exported from the nucleus to the cytoplasm in the miRNA biogenesis pathway?
(A) pre-miRNA
(B) pri-miRNA
(C) mature miRNA
(D) shRNA

2004

**78.** Peptide Nucleic Acids (PNAs) exhibit extremely high binding affinity to complementary DNA/RNA primarily because:
(A) They possess an uncharged pseudopeptide backbone lacking electrostatic repulsion
(B) They contain novel nucleobases with stronger hydrogen bonding
(C) They are highly positively charged
(D) They covalently cross-link to the target strand

2015

**79.** Antisense oligonucleotides (ASOs) can induce targeted gene silencing by all of the following mechanisms EXCEPT:
(A) Blocking ribosome binding
(B) Promoting RNase H-mediated degradation
(C) Altering pre-mRNA splicing
(D) Recruiting DNA polymerase for replication block

2009

**80.** Consider the following statements regarding morpholinos:
I. They have a phosphorodiamidate linkage instead of a phosphodiester bond.
II. They recruit RNase H to degrade target RNA.
III. They are highly resistant to nucleases.
IV. They function primarily by steric block mechanisms.
Which of the above statements is/are incorrect?
(A) I and III
(B) II
(C) II and IV
(D) I and IV

2005

**81.** In phage display technology, the exogenous peptide or protein is fused to:
(A) A phage coat protein (e.g., pIII or pVIII)
(B) The phage DNA polymerase
(C) The phage tail fibers
(D) The host cell receptor

2022

**82.** Which technique iteratively enriches for high-affinity binding phages from a phage display library?
(A) Biopanning
(B) Electroporation
(C) Blue-white screening
(D) Replica plating

2007

**83.** A single-chain variable fragment (scFv) consists of:
(A) A complete IgG molecule lacking the Fc region
(B) The variable regions of heavy (VH) and light (VL) chains connected by a flexible linker
(C) Only the heavy chain variable region (VH)
(D) Two Fab fragments joined together

2015

**84.** Humanized antibodies are primarily engineered to:
(A) Reduce immunogenicity when administered to humans
(B) Increase the molecular weight of the antibody
(C) Bind non-human antigens exclusively
(D) Eliminate the need for variable regions

2015

**85.** Consider the following statements regarding recombinant therapeutic insulin production:
I. It is produced by expressing the A and B

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2002

**105.** Ethical concerns regarding GMOs generally encompass all of the following EXCEPT:
(A) "Playing God" and interfering with nature
(B) Ensuring sequence accuracy of the inserted gene
(C) Potential allergenicity in GM foods
(D) Monopolization of the seed market by large corporations

2014

**106.** In UV spectrophotometry of nucleic acids, the $\frac{A_{260}}{A_{280}}$ ratio for highly pure RNA is approximately:
(A) 2.0
(B) 1.5
(C) 1.8
(D) 2.5

2021

**107.** Which statement accurately describes the denaturation kinetics of DNA?
(A) It involves a highly cooperative transition resulting in a sigmoidal melting curve
(B) It exhibits a broad, linear transition without cooperativity
(C) It requires specific exonucleases to separate strands
(D) It is an irreversible process

2025

**108.** The principle of separating plasmids from genomic DNA using cesium chloride (CsCl)-ethidium bromide density gradient centrifugation depends on:
(A) The difference in supercoiling, which restricts ethidium bromide intercalation in plasmids
(B) The difference in molecular weight between plasmids and genomic DNA
(C) The specific sequence of the origin of replication
(D) The presence of antibiotic resistance genes

2024

**109.** $T_4$ DNA ligase works most efficiently on which type of DNA termini?
(A) Cohesive (sticky) ends but can also ligate blunt ends
(B) Only 5' protruding cohesive ends
(C) RNA-DNA hybrid ends
(D) Only 3' protruding cohesive ends

2012

**110.** Consider the following statements concerning Type III restriction enzymes:
I. They require ATP and SAM for activity.
II. They cleave DNA precisely within the recognition sequence.
III. They possess both restriction and modification activities in a single complex.
Which of the above statements are correct?
(A) I and II
(B) I and III
(C) II and III
(D) I, II and III

2006

**111.** Which DNA modifying enzyme would be most suitable to remove protruding 3' overhangs to generate blunt ends?
(A) $T_4$ DNA polymerase (exonuclease activity)
(B) Klenow fragment
(C) Taq polymerase
(D) Alkaline phosphatase

2016

**112.** Why is Taq polymerase considered unsuitable for amplifying highly complex or extremely long genomic templates without modifications?
(A) It lacks 3' to 5' proofreading exonuclease activity
(B) It lacks 5' to 3' polymerase activity
(C) It degrades quickly at 72°C
(D) It only amplifies RNA

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2024

**113.** In automated Sanger sequencing, chain termination is achieved using:
(A) Dideoxynucleoside triphosphates (ddNTPs)
(B) Deoxynucleoside triphosphates (dNTPs)
(C) Ribonucleoside triphosphates (rNTPs)
(D) Methylated nucleotides

2010

**114.** The RNase H activity of reverse transcriptase is essential in cDNA library construction because it:
(A) Generates RNA fragments that serve as primers for the second strand synthesis
(B) Prevents the degradation of the mRNA template
(C) Adds poly-A tails to the newly formed cDNA
(D) Ligates cDNA fragments into the cloning vector

2010

**115.** A cDNA library differs fundamentally from a genomic library because a cDNA library:
(A) Represents only the genes that are actively transcribed in the source cells
(B) Contains both introns and exons
(C) Includes promoter and enhancer sequences

2013
**157.** Which enzyme strictly requires a DNA or RNA primer with a free 3'-OH group to initiate synthesis?
(A) DNA polymerase I
(B) RNA polymerase II
(C) $T_7$ RNA polymerase
(D) Poly(A) polymerase

2002
**158.** Type I restriction endonucleases are rarely used in cloning because:
(A) They cleave at random distances far from their recognition site
(B) They only cut single-stranded DNA
(C) They require extreme temperatures
(D) They do not recognize specific DNA sequences

2013
**159.** Neoschizomers are defined as restriction enzymes that:
(A) Recognize the same sequence but cleave at different positions
(B) Recognize different sequences but produce the same sticky ends
(C) Recognize the same sequence and cleave at the exact same position
(D) Recognize methylated DNA only

2017
**160.** Determine the correct sequence for PCR-based site-directed mutagenesis.
I. DpnI digestion
II. PCR amplification using mutagenic primers
III. Transformation into E. coli
IV. Primer design with mismatched base
Which of the following represents the correct sequence of steps?
(A) IV, II, I, III
(B) IV, I, II, III
(C) II, IV, I, III
(D) IV, II, III, I

2013
**161.** A reverse transcriptase (RT) lacking RNase H activity is highly desirable when synthesizing:
(A) Long, full-length cDNAs
(B) Short cDNA fragments
(C) RNA probes
(D) Genomic libraries

2009
**162.** Plasmids used as cloning vectors are often engineered to have a high copy number. This is typically achieved by mutating the:
(A) Origin of replication (e.g., rop gene mutation)
(B) Selectable marker gene
(C) Multiple cloning site
(D) Promoter sequence

2002
**163.** The maximum insert capacity of a standard replacement bacteriophage lambda vector is generally around:
(A) 20 kb
(B) 5 kb
(C) 100 kb
(D) 300 kb

2023
**164.** What is the main advantage of using a phagemid vector?
(A) It can be manipulated as a plasmid and packaged as single-stranded DNA upon helper phage infection
(B) It requires no host cell for propagation
(C) It integrates directly into the host genome
(D) It carries inserts up to 1000 kb

2013
**165.** The primary biological role of the naturally occurring F plasmid in E. coli, which forms the basis for BAC vectors, is:
(A) Conjugation and transfer of genetic material
(B) Resistance to heavy metals
(C) Production of restriction enzymes
(D) Synthesis of beta-lactamase

2015
**166.** Viral vectors constructed from adeno-associated viruses (AAV) are distinct because they:
(A) Integrate specifically at chromosome 19q13.4 in the human genome
(B) Replicate autonomously without any helper virus
(C) Elicit a massive immune response
(D) Have an enormous packaging capacity of 30 kb

2018
**167.** A well-known inducible promoter used in yeast expression systems (S. cerevisiae) is the:
(A) $GAL_1$ promoter
(B) AOX1 promoter
(C) Lac promoter
(D) CMV promoter

2003
**168.** Match the Expression Vector Component in List I with its specific function in List II.
List I: (Expression Vector Component)

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(B) I, II, IV and V
(C) II, III, IV and V
(D) I, III, IV and V

2003

**126.** Homopolymer tailing is an effective method for joining DNA molecules. If the vector is tailed with dGTP, the insert must be tailed with:
(A) dCTP
(B) dGTP
(C) dATP
(D) dTTP

2007

**127.** Microinjection of DNA into animal cells provides the highest transformation efficiency, but its major drawback is:
(A) It is extremely labor-intensive and low-throughput
(B) It requires the use of toxic chemicals
(C) It causes widespread immune responses
(D) It only works on dividing cells

2008

**128.** In plant biotechnology, the biolistic (gene gun) method is favored for transforming:
(A) Chloroplast genomes and recalcitrant monocots
(B) Only dicotyledonous plants
(C) Fungal protoplasts
(D) Agrobacterium-susceptible species exclusively

2007

**129.** Colony hybridization utilizes a labeled nucleic acid probe to identify recombinant clones. The specificity of this binding is dictated by:
(A) The stringency of the washing buffer (salt concentration and temperature)
(B) The specific activity of the radioactive label
(C) The type of restriction enzyme used
(D) The size of the bacterial colony

2011

**130.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Site-directed mutagenesis utilizing PCR often incorporates mismatched primers.
**Reason (R):** PCR entirely replaces the need for any host organism in the production of mutated proteins.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2023

**131.** Which component is strictly necessary for generating a targeted gene knockout in yeast via homologous recombination?
(A) A selectable marker flanked by sequences homologous to the target locus
(B) A viral capsid
(C) An RNA guide sequence
(D) An inducible promoter

2016

**132.** The process of inserting a functional gene into an organism to observe a gain-oF-function phenotype is commonly termed:
(A) Knockin
(B) Knockout
(C) Gene silencing
(D) Random mutagenesis

2009

**133.** During RNA interference (RNAi), what is the function of the passenger strand of the siRNA duplex?
(A) It is degraded or discarded from the RISC complex
(B) It binds to the target mRNA to initiate cleavage
(C) It acts as an RNA-dependent RNA polymerase
(D) It transports the RISC complex into the nucleus

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2024

**134.** Which organism fundamentally provided the first clear evidence of the RNAi mechanism (then called post-transcriptional gene silencing or cosuppression)?
(A) Petunia hybrida
(B) Caenorhabditis elegans
(C) Mus musculus
(D) Drosophila melanogaster

2018

**135.** What defines the key structural difference between a conventional antisense oligonucleotide (ASO) and a Peptide Nucleic Acid (PNA)?
(A) PNA lacks a sugar-phosphate backbone
(B) ASO is a double-stranded molecule
(C) PNA contains uracil instead of thymine
(D) ASO targets DNA while PNA targets RNA only

2022

**136.** Phage display allows the direct linkage of:
(A) A phenotype (the displayed protein) to its genotype (the encapsulated DNA)
(B) An RNA transcript to a ribosome
(C) A promoter to an enhancer
(D) An antibody to an antigen without a vector

2002

**137.** Which strategy is most commonly used to humanize a mouse monoclonal antibody?
(A) Grafting the mouse complementarity determining regions (CDRs) onto a human antibody framework
(B) Replacing the entire mouse heavy chain with a human light chain
(C) Fusing the mouse antibody to a human albumin protein
(D) Deleting the Fc region entirely

2007

**138.** Recombinant human growth hormone (somatotropin) produced in E. coli initially contained an extra amino acid at the N-terminus. Which amino acid was it?
(A) Methionine
(B) Valine
(C) Leucine
(D) Cysteine

2022

**139.** Subunit vaccines against Hepatitis B are most commonly produced by expressing the viral surface antigen (HBsAg) in:
(A) Saccharomyces cerevisiae
(B) Escherichia coli
(C) Mammalian cell culture
(D) Agrobacterium tumefaciens

2005

**140.** Match the Industrial Strain Improvement Strategy in List I with its definition in List II.
List I: (Strain Improvement Strategy)
P. Rational Design
Q. Protoplast Fusion
R. Mutagenesis
S. Directed Evolution
List II: (Definition)
1. Exposure to physical/chemical agents to generate random variants
2. Combining the genomes of two cells after removing their cell walls
3. Iterative rounds of random mutation and screening for specific traits
4. Targeted genetic modification based on detailed metabolic knowledge

(A) P-4, Q-2, R-1, S-3
(B) P-3, Q-2, R-1, S-4
(C) P-4, Q-1, R-2, S-3
(D) P-2, Q-4, R-3, S-1

2011

**141.** The primary purpose of metabolic engineering in industrial microbes is to:
(A) Reroute carbon fluxes to maximize the yield of a desired product
(B) Decrease the mutation rate of the host
(C) Enhance the pathogenicity of the strain
(D) Stop the production of secondary metabolites

2022

**142.** A significant challenge in producing Single Cell Protein (SCP) from bacterial sources for human consumption is the high content of:
(A) Nucleic acids
(B) Carbohydrates
(C) Saturated fats
(D) Cellulose

2002

**143.** Which characteristic of solid tumors makes them particularly suitable for targeted therapy using nano-delivery systems?
(A) Leaky vasculature and poor lymphatic drainage
(B) Low interstitial fluid pressure
(C) High pH environment
(D) Highly organized, non-porous vasculature

2005

**144.** Consider the following statements regarding genetically modified (GM) crops:
I. Bt cotton expresses a toxin from Bacillus thuringiensis.
II. Golden Rice is engineered to produce high levels of Vitamin $B_{12}$.
III. Herbicide-tolerant crops often carry a modified EPSPS gene.
IV. GM crops require evaluation by the GEAC in India before commercial release.
Which of the above statements is/are incorrect?
(A) I and II
(B) II
(C) III and IV
(D) I and IV

2014

**145.** A reporter gene is distinguished by its ability to:
(A) Produce a measurable signal indicating spatial or temporal gene expression
(B) Confer resistance to an antibiotic
(C) Destroy the host cell upon expression

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(D) Integrate non-specifically into the genome

2023

**146.** Which technique is most definitive for verifying the exact integration site of a transgene in a GM organism's genome?
(A) Genome walking (or Inverse PCR)
(B) Southern blotting
(C) Northern blotting
(D) ELISA

2010

**147.** Environmental Risk Assessment (ERA) of GMOs systematically evaluates "gene flow." Gene flow refers to:
(A) The transfer of transgenes to sexually compatible wild relatives
(B) The transcription rate of the transgene
(C) The movement of GMO seeds across national borders
(D) The horizontal transfer of genes to humans

2023

**148.** Biosafety Level 4 (BSL-4) facilities uniquely require:
(A) Positive pressure personnel suits with segregated air supply
(B) Use of standard lab coats and gloves
(C) Class II biological safety cabinets
(D) Open bench work

2005

**149.** The Cartagena Protocol on Biosafety is an international agreement primarily governing:
(A) The safe handling, transport, and use of living modified organisms (LMOs)
(B) The deposition of patented microbes
(C) The banning of all human cloning
(D) Intellectual property rights of indigenous plants

2008

**150.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Naturally occurring, unmodified genes discovered in the human genome cannot be patented in many jurisdictions.
**Reason (R):** They are considered products of nature and lack the required "inventive step."
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2023

**151.** Which factor most strongly affects the rate of renaturation ($Cot_{\frac{1}{2}}$) in DNA renaturation experiments?
(A) DNA sequence complexity
(B) Buffer pH
(C) Optical density at 260 nm
(D) Time of UV exposure

2008

**152.** DNA can be recovered from an agarose gel post-electrophoresis using:
(A) Silica-based spin columns and chaotropic salts
(B) Phenol-chloroform extraction
(C) Trichloroacetic acid precipitation
(D) Ammonium sulfate precipitation

2021

**153.** A genomic library constructed in BAC vectors is preferred over a cosmid library for whole-genome sequencing because it requires:
(A) Fewer clones to cover the entire genome
(B) A higher copy number in the host
(C) No restriction digestion
(D) Viral packaging

2019

**154.** What is the fundamental mechanism of electroporation in gene delivery?
(A) Formation of aqueous pores in the lipid bilayer via electrical pulses
(B) Permanent depolarization of the mitochondrial membrane potential
(C) Coating DNA with cationic lipids to facilitate cellular endocytosis
(D) Direct physical insertion of DNA into the cell using a micro-needle

2017

**155.** RNAi-mediated gene silencing is considered transient in non-dividing mammalian cells unless the shRNA is delivered via:
(A) A lentiviral vector
(B) Lipofectamine
(C) Electroporation
(D) A plasmid vector

2005

**156.** In producing recombinant therapeutic proteins, the term "inclusion bodies" refers to:
(A) Dense, insoluble aggregates of misfolded recombinant proteins in bacteria
(B) Organelles that secrete proteins
(C) Specialized vesicles for viral packaging
(D) Crystals used for X-ray crystallography

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(A) uidA (GUS)
(B) nptII (Kanamycin resistance)
(C) hpt (Hygromycin resistance)
(D) bar (Glufosinate resistance)

2007
**96.** What is the fundamental distinction between selectable and scorable marker genes?
(A) Selectable markers confer survival under toxic conditions, whereas scorable markers produce a visually identifiable phenotype.
(B) Scorable markers are only used in bacteria, whereas selectable markers are used in plants.
(C) Selectable markers involve fluorescence, whereas scorable markers provide antibiotic resistance.
(D) Scorable markers integrate into the host genome, but selectable markers do not.

2023
**97.** The term "transgenic event" refers strictly to:
(A) The unique insertion of a specific recombinant DNA construct into a specific genomic location
(B) The protocol used to transform the organism
(C) The day the organism expresses the transgene
(D) The commercial release of a GMO

2021
**98.** Biosafety level 3 (BSL-3) containment is specifically mandated for working with agents that:
(A) Cause severe or lethal disease primarily via aerosol transmission
(B) Pose minimal potential hazard to personnel and the environment
(C) Represent the highest risk of life-threatening disease with no available vaccines
(D) Are non-pathogenic industrial strains

2015
**99.** Which Indian regulatory body is responsible for approving the environmental release of genetically modified crops?
(A) Recombinant DNA Advisory Committee (RDAC)
(B) Review Committee on Genetic Manipulation (RCGM)
(C) Genetic Engineering Appraisal Committee (GEAC)
(D) Institutional Biosafety Committee (IBSC)

2010
**100.** Match the Indian Regulatory Body in List I with its primary mandate in List II.

List I: (Regulatory Body)
P. IBSC
Q. RCGM
R. GEAC
S. DLC

List II: (Mandate)
1. Monitors local node experiments at the institutional level
2. Grants approval for large-scale field trials and commercialization
3. Reviews small-scale confined field trials
4. District-level compliance and monitoring

(A) P-1, Q-3, R-2, S-4
(B) P-1, Q-2, R-3, S-4
(C) P-4, Q-3, R-2, S-1
(D) P-3, Q-1, R-4, S-2

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2017
**101.** An environmental risk assessment of a GMO evaluates which primary ecological concern?
(A) Economic impact on traditional farmers
(B) Potential for transgene flow to wild relatives
(C) Patent rights associated with the inserted gene
(D) Public acceptance of the GM product

2023
**102.** For an invention to be patentable, it must definitively satisfy three criteria: novelty, industrial applicability, and:
(A) Non-obviousness (inventive step)
(B) High monetary value
(C) Natural occurrence
(D) Immediate commercialization

2001
**103.** The Budapest Treaty facilitates international patent processes primarily by standardizing the rules for:
(A) The deposition of microorganisms in recognized culture collections
(B) The ethical treatment of laboratory animals
(C) Biosafety containment levels across borders
(D) Tariffs on biotechnological imports

2008
**104.** Under the TRIPS Agreement, countries must provide patent protection for inventions in all fields of technology, provided they are:
(A) Related to national defense
(B) New, involve an inventive step, and are capable of industrial application
(C) Pertaining only to non-living matter
(D) Exclusively beneficial to public health

P. Ori
Q. Promoter
R. Selectable marker
S. His-tag

List II: (Function)

1. Drives transcription of the insert
2. Facilitates one-step protein purification
3. Ensures autonomous replication of the vector
4. Allows survival of host cells containing the plasmid

(A) P-3, Q-1, R-4, S-2
(B) P-1, Q-3, R-2, S-4
(C) P-3, Q-4, R-1, S-2
(D) P-4, Q-2, R-1, S-3

2004

**169.** Linkers are distinctively characterized as:
(A) Short, blunt-ended, double-stranded synthetic DNA containing a restriction site
(B) Long, single-stranded RNA molecules
(C) Proteins that connect DNA strands
(D) Natural repetitive DNA sequences

2021

**170.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).

**Assertion (A):** In vitro viral packaging drastically increases the efficiency of lambda vector transformation compared to chemical transformation.

**Reason (R):** Viral packaging protects the recombinant DNA from degradation and actively injects it into the host cell via natural infection pathways.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2003

**171.** Lipofection achieves gene delivery primarily via:
(A) Endocytosis of lipid-DNA complexes
(B) Viral receptor-mediated entry
(C) Creation of electrical pores
(D) Micro-projectile bombardment

2023

**172.** Insertional inactivation of the lacZ alpha peptide gene results in colonies that appear:
(A) White on X-gal plates
(B) Blue on X-gal plates
(C) Red on MacConkey agar
(D) Fluorescent green under UV

2015

**173.** Primer extension mutagenesis typically employs:
(A) Primers containing the desired mutation flanked by complementary sequences
(B) Random hexamer primers
(C) Primers with complete homology to the template
(D) Oligo(dT) primers

2010

**174.** Which process relies on homologous recombination to replace a wild-type gene with a non-functional copy?
(A) Targeted gene disruption (Knockout)
(B) Gene knockdown
(C) Directed evolution
(D) Transfection

2005

**175.** The fundamental role of the TRBP protein in the human RNAi pathway is to:
(A) Assist Dicer and recruit the $AgO_2$ protein to form the RISC
(B) Synthesize double-stranded RNA
(C) Export pre-miRNA from the nucleus
(D) Degrade single-stranded mRNA independently

2019

**176.** Which characteristic of Morpholino oligonucleotides makes them particularly suitable for in vivo gene silencing?
(A) They are uncharged and highly resistant to nucleolytic degradation
(B) They are rapidly degraded by cellular nucleases
(C) They carry a strong negative charge
(D) They trigger massive immune responses

2018

**177.** The production of completely humanized antibodies from mice requires the transgenic integration of:
(A) Entire human immunoglobulin gene loci into a mouse whose endogenous loci are inactivated
(B) Mouse variable regions and human constant regions
(C) Human T-cells into the mouse immune system
(D) Mouse constant regions into human cell lines

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(C) Herbivores
(D) Drought stress

2012
**271.** An antibody's specific antigen-binding capability is strictly determined by its:
(A) Complementarity Determining Regions (CDRs) in the variable domains
(B) Constant heavy regions
(C) Fc region
(D) Disulfide bonds

2021
**272.** A biosafety cabinet Class II provides protection for:
(A) Both the personnel and the product
(B) The personnel only
(C) The product only
(D) Neither personnel nor product, only the environment

2006
**273.** The "inventive step" requirement for a patent means the invention must:
(A) Not be obvious to a person having ordinary skill in the art
(B) Be mathematically proven
(C) Be completely discovered in nature
(D) Cost a large amount to develop

2023
**274.** In organic extraction of nucleic acids, treating the sample with a phenol:chloroform:isoamyl alcohol (25:24:1) mixture specifically relies on:
(A) Differences in solubility of molecules between aqueous and organic phases
(B) Size exclusion principles
(C) Ionic binding to a resin
(D) Specific antibody binding

2005
**275.** Polynucleotide kinase transfers a phosphate from ATP. Which phosphate of ATP is specifically transferred?
(A) Gamma
(B) Alpha
(C) Beta
(D) Delta

2016
**276.** Which condition strongly promotes complete DNA renaturation (annealing)?
(A) Incubation at $T_m - 25°C$ with moderate to high salt concentration
(B) Boiling temperature and low salt
(C) Highly basic pH (>12)
(D) Addition of strong chaotropic agents

2013
**277.** The resolving power of a polyacrylamide gel is primarily determined by:
(A) The ratio and concentration of acrylamide to bisacrylamide
(B) The voltage applied
(C) The pH of the running buffer
(D) The amount of DNA loaded

2025
**278.** Reverse transcriptase is fundamentally a:
(A) RNA-dependent DNA polymerase
(B) DNA-dependent RNA polymerase
(C) RNA-dependent RNA polymerase
(D) Ribozyme

2004
**279.** Which element is generally NOT required in an E. coli cloning vector if protein expression is not the goal?
(A) Strong promoter
(B) Origin of replication
(C) Selectable marker
(D) Multiple cloning site

2016
**280.** When constructing a cDNA library, why might one use adapters rather than blunt-end ligation?
(A) Adapters provide cohesive ends, which ligate with significantly higher efficiency
(B) Blunt-end ligation is impossible with cDNA
(C) Adapters add poly-A tails automatically
(D) Adapters act as primers for replication

2007
**281.** The alpha-complementation phenomenon relies on the interaction between:
(A) A defective beta-galactosidase encoded by the host and a small peptide encoded by the plasmid
(B) Two separate antibiotic resistance genes
(C) $T_4$ DNA ligase and the cloning vector
(D) RNA polymerase and an inducible promoter

2011
**282.** During site-directed mutagenesis utilizing a plasmid vector, the DpnI enzyme is used to selectively destroy the wild-type template because DpnI strictly targets:
(A) Methylated DNA (specifically GmATC sequences)
(B) Unmethylated DNA
(C) Single-stranded DNA
(D) RNA-DNA hybrids

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2010
**189.** A YAC vector replicates in yeast. To be a true shuttle vector, it must also possess which elements for propagation in E. coli?
(A) $ColE_1$ origin and ampicillin resistance gene
(B) ARS and CEN sequences
(C) $LEU_2$ and $URA_3$ genes
(D) Telomeres

2012
**190.** Adenoviruses have a genome composed of:
(A) Linear double-stranded DNA
(B) Single-stranded RNA
(C) Double-stranded RNA
(D) Circular double-stranded DNA

2021
**191.** A key limitation of conventional retroviral vectors in gene therapy is their propensity to:
(A) Cause insertional mutagenesis by integrating near proto-oncogenes
(B) Trigger immense acute inflammation
(C) Fail to integrate into host DNA
(D) Degrade within hours of delivery

2003
**192.** Which yeast species is highly favored for recombinant protein production due to its ability to perform high-density fermentation and secrete proteins efficiently?
(A) Pichia pastoris
(B) Saccharomyces cerevisiae
(C) Schizosaccharomyces pombe
(D) Candida albicans

2008
**193.** The GST tag enables purification of recombinant proteins using affinity chromatography with:
(A) Glutathione sepharose
(B) Nickel-NTA agarose
(C) Amylose resin
(D) Protein A

2014
**194.** In vitro viral packaging of lambda phage recombinants specifically requires:
(A) Empty phage heads and tail assemblies
(B) Helper plasmids
(C) Reverse transcriptase
(D) Intact host bacterial cells

2004
**195.** Which chemical effectively permeabilizes E. coli membranes during the preparation of competent cells?
(A) $CaCl_2$
(B) $MgSO_4$
(C) NaCl
(D) KCl

2017
**196.** Consider the following statements about blue-white screening:
I. The host cell must have a deletion in the lacZ gene ($lacZ\Delta M_{15}$).
II. IPTG is used as an inducer for the lac promoter.
III. X-gal is cleaved to form a blue precipitate.
IV. Recombinant colonies appear blue.
Which of the above statements are correct?
(A) I, II and III
(B) II, III and IV
(C) I, III and IV
(D) I, II and IV

2004
**197.** Chemical mutagenesis utilizing EMS is characterized primarily by generating:
(A) Random point mutations via guanine alkylation
(B) Large genomic deletions
(C) Frameshift mutations
(D) Transposon insertions

2011
**198.** RNAi is technically categorized as a form of:
(A) Post-transcriptional gene silencing
(B) Transcriptional gene silencing
(C) Post-translational modification
(D) Pre-transcriptional gene editing

2015
**199.** Synthetic siRNAs introduced into mammalian cells typically consist of:
(A) 21-23 bp double-stranded RNA with 2-nucleotide 3' overhangs
(B) 50-100 bp double-stranded RNA
(C) Single-stranded RNA of 15 bases
(D) Circular single-stranded RNA

2012
**200.** The primary advantage of a single-chain variable fragment (scFv) over a whole monoclonal antibody is its:
(A) Smaller size allowing better tissue penetration
(B) Ability to trigger the complement cascade
(C) Longer halF-life in the bloodstream
(D) Higher avidity due to multiple binding sites

2023
**201.** Which method is most scalable for the mass production of therapeutic monoclonal antibodies?

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2008
**178.** What was the primary safety issue associated with extracting human growth hormone from cadaveric pituitary glands prior to the advent of recombinant technology?
(A) Risk of transmitting Creutzfeldt-Jakob disease (prions)
(B) Contamination with HIV
(C) Severe allergic reactions to non-human proteins
(D) High levels of endotoxin

2019
**179.** Polymeric nanocarriers such as PLGA (poly(lactic-co-glycolic acid)) are highly valued in drug delivery because they are:
(A) Biocompatible and biodegrade into naturally occurring metabolic products
(B) Completely non-biodegradable
(C) Highly toxic to tumor cells directly
(D) Composed of purely inorganic materials

2021
**180.** Match the Genetically Modified Organism in List I with its engineered trait in List II.
List I: (GMO)
P. Flavr Savr Tomato
Q. Roundup Ready Soybean
R. Bt Cotton
S. Golden Rice
List II: (Trait)
1. Resistance to the herbicide glyphosate
2. Insect resistance via Cry proteins
3. Enhanced provitamin A (beta-carotene) biosynthesis
4. Delayed ripening via antisense polygalacturonase
(A) P-4, Q-1, R-2, S-3
(B) P-1, Q-4, R-2, S-3
(C) P-4, Q-2, R-1, S-3
(D) P-3, Q-1, R-4, S-2

2003
**181.** A critical principle of the TRIPS agreement is that patents must be valid for a minimum period of:
(A) 20 years
(B) 5 years
(C) 10 years
(D) 50 years

2012
**182.** Extraction of pure intact high molecular weight genomic DNA requires avoiding:
(A) Vigorous vortexing or physical shearing
(B) Proteinase K
(C) SDS
(D) RNase A

2001
**183.** Identify the enzyme that generates cohesive 3' protruding ends upon DNA cleavage.
(A) PstI
(B) EcoRI
(C) SmaI
(D) HindIII

2012
**184.** Which chemical is often used in plant tissue culture to facilitate protoplast fusion?
(A) Polyethylene glycol (PEG)
(B) Sodium chloride
(C) Hydrochloric acid
(D) Ethanol

2009
**185.** Reverse transcription is characterized by all of the following enzymatic activities EXCEPT:
(A) RNA-dependent RNA polymerase
(B) RNA-dependent DNA polymerase
(C) DNA-dependent DNA polymerase
(D) RNase H

2017
**186.** Which sequence is an example of a typical Type II restriction enzyme recognition site (palindrome)?
(A) 5'-GAATTC-3'
(B) 5'-GATCAG-3'
(C) 5'-AAAAAA-3'
(D) 5'-ATGCAG-3'

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2005
**187.** In an agarose gel, the migration rate of linear double-stranded DNA is inversely proportional to the:
(A) Logarithm of its molecular weight
(B) Voltage applied
(C) Buffer concentration
(D) EtBr concentration

2025
**188.** Standard PCR typically cannot amplify fragments larger than 5-10 kb efficiently. Long-range PCR overcomes this by utilizing:
(A) A mixture of Taq polymerase and a proofreading polymerase
(B) Extremely high temperatures (98°C) for extension
(C) RNA primers instead of DNA primers
(D) Very high magnesium concentrations

2008

**213.** A cosmid vector fundamentally acts as a plasmid but accommodates large DNA fragments because it:
(A) Is packaged into phage heads in vitro via its cos sites
(B) Exists strictly as a linear molecule in E. coli
(C) Carries multiple origins of replication
(D) Prevents host cell division

2002

**214.** Which vector is engineered to strictly maintain a single copy number per host cell to increase the stability of extremely large inserts?
(A) BAC
(B) $pUC_{19}$
(C) YAC
(D) Phagemid

2016

**215.** What is the fundamental role of the helper virus in an AAV-based gene delivery system?
(A) It provides proteins necessary for AAV replication
(B) It encapsulates the AAV DNA
(C) It acts as the therapeutic agent
(D) It silences the host immune system

2017

**216.** Match the Mutagenesis Technique in List I with its defining feature in List II.
List I: (Mutagenesis Technique)
P. Chemical mutagenesis
Q. Cassette mutagenesis
R. PCR site-directed mutagenesis
S. DNA shuffling
List II: (Defining Feature)
1. Introduces mutations globally via agents like EMS
2. Amplifies template with mismatched primers
3. Recombines fragmented homologous genes to evolve new functions
4. Replaces a restriction fragment with a synthetic mutant duplex
(A) P-1, Q-4, R-2, S-3
(B) P-4, Q-1, R-2, S-3
(C) P-1, Q-2, R-4, S-3
(D) P-2, Q-4, R-3, S-1

2015

**217.** Shuttle vectors constructed for Saccharomyces cerevisiae commonly use the $URA_3$ gene as a:
(A) Selectable marker complementing a host auxotrophy
(B) Constitutive promoter
(C) Terminator
(D) Centromere

2001

**218.** An inducible promoter allows researchers to:
(A) Decouple the growth phase of the culture from the protein production phase
(B) Permanently alter the host genome
(C) Remove introns from the transcript
(D) Bypass translation

2017

**219.** Consider the following statements concerning Northern blotting:
I. It is used to detect specific RNA sequences.
II. RNA is separated by electrophoresis prior to transfer.
III. It utilizes antibodies as probes.
IV. It provides information on transcript size and abundance.
Which of the above statements are correct?
(A) I, II and IV
(B) II, III and IV
(C) I and II
(D) I, III and IV

2012

**220.** Calcium phosphate transfection facilitates DNA uptake by:
(A) Forming a DNA-calcium complex precipitate that adheres to the cell and is endocytosed
(B) Directly permeabilizing the nuclear envelope
(C) Applying an electrical field
(D) Acting as a viral envelope

2007

**221.** Which method provides the quickest preliminary confirmation that a bacterial colony contains a plasmid with the desired insert?
(A) Colony PCR
(B) Southern blotting
(C) Next-generation sequencing
(D) Microarray analysis

2019

**222.** An organism carrying a completely inactivated specific gene is functionally designated a:
(A) Knockout
(B) Transgenic
(C) Knockin
(D) Chimera

2017

**223.** What endogenous pathway does artificial RNA interference (RNAi) exploit to silence target genes?
(A) The microRNA/siRNA regulatory pathway

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(B) CRISPR-Cas immunity
(C) Nonsense-mediated decay
(D) Ubiquitin-proteasome system

2012
**224.** Antisense RNA blocks gene expression primarily at which stage?
(A) RNA processing and translation
(B) Transcription initiation
(C) Post-translational modification
(D) DNA replication

2023
**225.** A humanized antibody generally retains what percentage of mouse-derived protein sequence?
(A) ~5-10% (only the CDRs)
(B) 100%
(C) ~50%
(D) 0%

2008
**226.** Recombinant factor VIII, produced in mammalian cells, is clinically crucial for treating:
(A) Hemophilia A
(B) Diabetes mellitus
(C) Pituitary dwarfism
(D) Multiple sclerosis

2013
**227.** In the context of industrial microbiology, what does "strain improvement" NOT typically aim to achieve?
(A) Reversion to wild-type characteristics
(B) Resistance to phage infections
(C) Increased yield of the target metabolite
(D) Tolerance to higher product concentrations

2025
**228.** Lipid nanoparticles (LNPs) structurally consist of:
(A) An aqueous core surrounded by a lipid bilayer or a solid lipid core
(B) A purely proteinaceous capsid
(C) A solid metallic core coated with lipids
(D) A network of cross-linked carbohydrates

2023
**229.** The nptII gene, widely used in plant transgenesis, confers resistance to:
(A) Kanamycin
(B) Glyphosate
(C) Hygromycin
(D) Bialaphos

NODIA

2017
**230.** Transgenic plants expressing the Bacillus thuringiensis (Bt) Cry proteins are specifically resistant to:
(A) Lepidopteran insect pests
(B) Fungal infections
(C) Viral diseases
(D) Broad-spectrum herbicides

2021
**231.** The primary purpose of containment protocols in recombinant DNA research is to:
(A) Prevent the escape and establishment of genetically modified agents in the environment
(B) Ensure exclusive access to intellectual property
(C) Protect laboratory equipment from contamination
(D) Standardize the genetic code

2025
**232.** Which of the following is NOT a patentable subject matter under typical international IP laws?
(A) A newly discovered naturally occurring rainforest plant
(B) A novel genetically engineered bacterium
(C) A new process for synthesizing DNA
(D) A specific, synthesized vector sequence

2013
**233.** The optical density (OD) of a double-stranded DNA solution is measured at 260 nm. An OD260 of 1.0 roughly corresponds to a DNA concentration of:
(A) 50 µg/mL
(B) 33 µg/mL
(C) 40 µg/mL
(D) 100 µg/mL

2005
**234.** Formamide is often added to DNA solutions to lower the melting temperature ($T_m$). It achieves this by:
(A) Disrupting hydrogen bonds between base pairs
(B) Covalently cross-linking strands
(C) Digesting the sugar-phosphate backbone
(D) Increasing the ionic strength

2022
**235.** Restriction enzyme digestion maps are constructed primarily by analyzing:
(A) The sizes of DNA fragments produced after single and double digests

(B) The sequence of the enzyme
(C) The GC content of the DNA
(D) The transcription rate of the DNA

2010
**236.** DNA polymerases universally catalyze chain elongation in which direction?
(A) 5' to 3' only
(B) 3' to 5' only
(C) Both 5' to 3' and 3' to 5'
(D) Randomly based on the template

2022
**237.** Real-time quantitative PCR (qPCR) differs from standard PCR primarily because qPCR:
(A) Allows monitoring of amplification during the exponential phase using fluorescent probes
(B) Uses RNA instead of DNA
(C) Requires no thermocycling
(D) Is less sensitive

2005
**238.** In cloning, the term "directional cloning" refers to:
(A) Using two different incompatible restriction enzymes to ensure the insert ligates in only one specific orientation
(B) Inserting DNA at random sites
(C) Using a single blunt-cutting enzyme
(D) Ligating RNA into a DNA vector

2009
**239.** The $T_7$ promoter in pET expression vectors requires the presence of which specific enzyme in the host cell for transcription?
(A) $T_7$ RNA polymerase
(B) E. coli RNA polymerase
(C) Reverse transcriptase
(D) $T_4$ DNA ligase

2014
**240.** The process of generating a genomic library typically involves partial digestion of the genome. Why is partial digestion preferred over complete digestion?
(A) To generate a set of overlapping fragments ensuring representation of the entire genome
(B) To produce solely extremely small fragments
(C) To strictly avoid cutting inside gene sequences
(D) To speed up the enzymatic reaction

2001
**241.** Identify the incorrect statement about electroporation.
(A) It requires the DNA solution to have high salt concentrations.
(B) It can be used for both bacteria and eukaryotic cells.
(C) It involves applying high-voltage electrical pulses.
(D) It creates transient pores in the cell membrane.

2016
**242.** Green Fluorescent Protein (GFP) is an excellent reporter gene primarily because:
(A) It emits fluorescence directly upon UV/blue light excitation without needing exogenous substrates
(B) It requires a specific substrate to be added for detection
(C) It confers resistance to ampicillin
(D) It directly enhances transcription rates

2019
**243.** In site-directed mutagenesis via the primer extension method, what dictates the location of the mutation?
(A) The sequence of the mutagenic oligonucleotide primer
(B) The restriction enzyme used
(C) The DNA ligase
(D) The host cell's repair machinery

2007
**244.** The RISC complex is central to RNAi. What does RISC stand for?
(A) RNA-Induced Silencing Complex
(B) RNA-Initiated Synthesis Complex
(C) Ribosome-Interfering Small Complex
(D) Rapid Internal Splicing Complex

NODIA

2015
**245.** Compared to standard RNA duplexes, locked nucleic acids (LNAs) used in gene silencing offer the advantage of:
(A) Exceptionally high thermal stability ($T_m$) and nuclease resistance
(B) Faster degradation in serum
(C) Increased conformational flexibility
(D) Inability to bind to DNA

2004
**246.** Phage display technology relies heavily on the ability of E. coli to:
(A) Continually secrete filamentous phages (like $M_{13}$) without lysing the cell
(B) Form spores
(C) Produce restriction endonucleases
(D) Ferment lactose

2010
**247.** Monoclonal antibodies are defined as antibodies that:
(A) Are produced from a single B-cell clone and recognize a single specific epitope
(B) Bind to multiple different epitopes on a single antigen
(C) Are always produced in plants
(D) Consist only of heavy chains

2002
**248.** E. coli is generally poor at producing complex human therapeutic proteins because it cannot:
(A) Perform sophisticated post-translational modifications like glycosylation
(B) Translate human mRNA
(C) Replicate plasmid vectors
(D) Grow in large fermenters

2003
**249.** Systemic delivery of naked plasmid DNA for gene therapy is typically highly inefficient due to:
(A) Rapid clearance and degradation by serum nucleases
(B) Excessive integration into the genome
(C) Immediate transcription in the blood
(D) Spontaneous mutations in the plasma

2017
**250.** Selectable marker genes in plant transgenesis are typically driven by which type of promoter to ensure consistent selection?
(A) A strong, constitutive promoter (e.g., CaMV $_{35}$S)
(B) A tissue-specific promoter
(C) An inducible promoter
(D) A late-stage viral promoter

2017
**251.** The environmental release of Bt Brinjal in India was subjected to an indefinite moratorium primarily due to:
(A) Concerns from the public and scientists regarding comprehensive safety testing and biodiversity impact
(B) Its inability to resist pests
(C) Lack of intellectual property rights
(D) Failure of the gene to express in the fruit

2022
**252.** The term "prior art" in patent law refers to:
(A) All information that has been disclosed to the public in any form before a given date
(B) The artistic design of the invention
(C) Patents that have already expired
(D) The initial draft of a patent application

2015
**253.** Which chemical principle underlies the purification of DNA via silica columns?
(A) DNA binds to silica in the presence of high concentrations of chaotropic salts
(B) DNA binds to silica only at high pH
(C) DNA repels silica but proteins bind tightly
(D) DNA forms covalent bonds with the silica matrix

2010
**254.** When designing a PCR primer, it is generally recommended to have a "GC clamp" at the 3' end. This refers to:
(A) Presence of one or two G or C bases at the 3' end to promote specific binding
(B) A stretch of 10 GC pairs
(C) Avoiding G and C altogether
(D) A chemical x-linking agent

2015
**255.** In the traditional Maxam-Gilbert DNA sequencing method, sequence reading is based on:
(A) Chemical modification and specific cleavage of DNA bases
(B) Chain termination with ddNTPs
(C) Pyrophosphate release during synthesis
(D) pH changes during synthesis

2015
**256.** Which RNA polymerase is routinely used to generate large amounts of RNA transcripts in vitro from a cloned DNA insert?
(A) $SP_6$ or $T_7$ RNA polymerase
(B) E. coli RNA polymerase
(C) Taq polymerase
(D) Reverse transcriptase

2010
**257.** An expression vector intended to secrete the recombinant protein in E. coli must include:
(A) An N-terminal signal sequence targeting the Sec or Tat pathway
(B) A poly-A tail
(C) A nuclear localization signal
(D) A centromere

2008
**258.** Viral packaging limits for bacteriophage lambda ensure that only recombinants containing an insert of the correct size are viable. This is a form of:
(A) Physical sizing selection

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(B) Positive selection
(C) Negative selection
(D) Alpha-complementation

2005
**259.** Southern blotting fundamentally involves the transfer of which macromolecule to a membrane?
(A) DNA
(B) RNA
(C) Protein
(D) Lipids

2007
**260.** The primary goal of generating a targeted gene knockin is to:
(A) Introduce a specific mutation or a reporter gene precisely at the endogenous locus
(B) Eliminate the expression of a specific gene entirely
(C) Randomly mutate the entire genome
(D) Silence RNA post-transcriptionally

2003
**261.** MicroRNAs (miRNAs) usually bind to which region of the target mRNA to exert their repressive effect?
(A) 3' Untranslated Region (3' UTR)
(B) 5' Untranslated Region (5' UTR)
(C) Coding sequence (CDS)
(D) Introns

2019
**262.** Recombinant DNA technology has enabled the production of tissue plasminogen activator (tPA), which is clinically used to:
(A) Dissolve blood clots in patients with stroke or myocardial infarction
(B) Treat hemophilia
(C) Promote cell growth
(D) Act as a vaccine against viruses

2003
**263.** Golden Rice is a genetically modified crop designed specifically to combat global deficiencies in:
(A) Vitamin A
(B) Iron
(C) Vitamin C
(D) Protein

2014
**264.** According to TRIPS, compulsory licensing allows a government to:
(A) Permit someone else to produce the patented product without the consent of the patent owner under specific circumstances
(B) Grant perpetual patents
(C) Ban the patenting of all biotechnological products
(D) Waive all patent fees globally

2021
**265.** What distinguishes a DNA sequence as a palindrome in the context of restriction enzymes?
(A) It reads the same 5' to 3' on both complementary strands
(B) It consists entirely of AT base pairs
(C) It alternates purines and pyrimidines perfectly
(D) It forms a cloverleaf structure

2011
**266.** A critical property of the Taq DNA polymerase used in standard PCR is that it lacks:
(A) 3' to 5' proofreading exonuclease activity
(B) Thermostability
(C) 5' to 3' exonuclease activity
(D) 5' to 3' polymerase activity

2017
**267.** The main purpose of the multiple cloning site (MCS) in a plasmid vector is to:
(A) Provide a cluster of unique restriction enzyme recognition sites for insert insertion
(B) Provide an origin of replication
(C) Confer antibiotic resistance
(D) Serve as a promoter for transcription

2005
**268.** Which technique evaluates the physical interaction between a specific protein and a DNA sequence?
(A) Electrophoretic Mobility Shift Assay (EMSA)
(B) Northern Blot
(C) Southern Blot
(D) SDS-PAGE

NODIA

2025
**269.** The Ti plasmid causes which disease in susceptible plants?
(A) Crown gall disease
(B) Leaf rust
(C) Powdery mildew
(D) Root knot

2015
**270.** RNA interference in plants is an evolutionary adaptation thought to have developed primarily as a defense against:
(A) RNA viruses
(B) Fungal pathogens

(A) Culturing hybridoma cells in stirred-tank bioreactors
(B) Extraction from immunized animal serum
(C) Expression in E. coli
(D) Phage display in culture

2011
**202.** Single Cell Protein (SCP) typically refers to edible biomass derived from:
(A) Microalgae, fungi, or bacteria
(B) Animal cell cultures
(C) Plant tissue cultures
(D) Genetically modified fish

2003
**203.** The term "stealth liposomes" refers to lipid nanoparticles that have been surface-modified with:
(A) Polyethylene glycol (PEG)
(B) Antibodies
(C) Cholesterol
(D) Viral glycoproteins

2006
**204.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Agrobacterium tumefaciens is widely used to transform dicotyledonous plants.
**Reason (R):** Dicotyledonous plants naturally secrete phenolic compounds like acetosyringone upon wounding, which activate vir genes.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2024
**205.** The GEAC (Genetic Engineering Appraisal Committee) in India operates under which ministry?
(A) Ministry of Environment, Forest and Climate Change
(B) Ministry of Agriculture
(C) Ministry of Science and Technology
(D) Ministry of Health and Family Welfare

2004
**206.** According to the Indian Patent Act, plants and animals in whole or any part thereof (other than microorganisms) are:
(A) Not patentable
(B) Patentable if genetically modified
(C) Patentable only if completely synthetic
(D) Patentable under a 10-year term

2024
**207.** Which statement accurately describes RNA denaturation?
(A) It eliminates secondary structures like hairpins
(B) It permanently destroys the RNA primary sequence
(C) It requires breaking covalent bonds
(D) It only occurs at temperatures above 100°C

2013
**208.** During plasmid purification, RNase A is often added to the resuspension buffer. To prevent DNase contamination, the RNase A should first be:
(A) Boiled
(B) Frozen at -80°C
(C) Treated with EDTA
(D) Dialyzed

2018
**209.** DNA polymerase I from E. coli possesses multiple catalytic activities. The Klenow fragment lacks which specific activity?
(A) 5' to 3' exonuclease
(B) 5' to 3' polymerase
(C) 3' to 5' exonuclease
(D) Endonuclease

2018
**210.** Isoschizomers that cleave at the exact same position within the identical recognition site will generate:
(A) Compatible sticky ends
(B) Incompatible sticky ends
(C) Blunt ends only
(D) Varying methylation patterns

2013
**211.** Which dye is replacing ethidium bromide as a safer alternative for DNA visualization in gels?
(A) SYBR Green
(B) Bromophenol blue
(C) Coomassie Brilliant Blue
(D) Xylene cyanol

2006
**212.** To synthesize a highly specific, full-length cDNA corresponding to a single known mRNA sequence, which primer is best?
(A) Gene-specific primer
(B) Oligo(dT) primer
(C) Random hexamer primer
(D) Poly-A primer

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2004
**283.** In mammals, long double-stranded RNA (> 30 bp) is generally not used for experimental RNAi because it triggers:
(A) An interferon response leading to global translation shutdown
(B) Immediate cell division
(C) Integration into the genome
(D) Random mutation of host genes

2004
**284.** Recombinant insulin is considered superior to animal-derived insulin primarily because it:
(A) Is identical to human insulin, thereby eliminating allergic reactions
(B) Has a completely different amino acid sequence
(C) Does not require refrigeration
(D) Is significantly cheaper to extract from natural sources

2021
**285.** In industrial biotechnology, scaling up a fermentation process from a shake flask to a bioreactor is fraught with challenges primarily related to:
(A) Maintaining adequate aeration, mixing, and heat transfer
(B) Gene sequence mutations
(C) The disappearance of promoters
(D) Excessive sterility

2015
**286.** Liposomes used in systemic drug delivery are often criticized for their rapid clearance. This is typically mitigated by:
(A) Coating them with polyethylene glycol (PEG)
(B) Decreasing their size to under 1 nm
(C) Making them highly positively charged
(D) Constructing them entirely from proteins

2008
**287.** Agrobacterium tumefaciens facilitates gene transfer into plant cells via:
(A) A Type IV secretion system that transfers the T-DNA complex
(B) A viral vector
(C) Direct microinjection
(D) Electroporation

2006
**288.** In India, final approval for the commercial release of a genetically modified crop must be granted by the:
(A) GEAC
(B) IBSC
(C) RCGM
(D) Ministry of Agriculture alone

2001
**289.** The term "Biopiracy" primarily describes:
(A) The unauthorized use or patenting of biological resources and traditional knowledge without fair compensation
(B) The theft of laboratory equipment
(C) Pirating software used in bioinformatics
(D) Selling GM crops illegally

2008
**290.** The process of converting RNA into cDNA before performing a PCR reaction is termed:
(A) RT-PCR
(B) Real-Time PCR
(C) Multiplex PCR
(D) Nested PCR

NODIA

2013
**291.** What is the primary role of formamide in nucleic acid hybridization buffers?
(A) To lower the melting temperature of double-stranded nucleic acids
(B) To act as a strong detergent
(C) To provide the necessary radioactive signal
(D) To precipitate the DNA

2024
**292.** Retroviruses encode an integrase enzyme. What is its primary function?
(A) It catalyzes the insertion of the viral DNA into the host genome
(B) It cuts the viral RNA into small pieces
(C) It synthesizes the viral envelope
(D) It degrades host cellular DNA

2019
**293.** Which enzyme is typically used in the first step of DNA isolation from bacterial cells to digest the peptidoglycan cell wall?
(A) Lysozyme
(B) Proteinase K
(C) RNase A
(D) DNA polymerase

2007
**294.** In creating a genomic library, what is the role of bacteriophage packaging extracts?
(A) They assemble mature phage particles around the recombinant cosmid or lambda DNA
(B) They randomly shear the genomic DNA
(C) They replicate the DNA inside the test tube

cellular RNA remains intact while other cellular components are solubilized, providing a reliable environment for extracting high-quality, full-length transcripts for analysis.

**10.** (A) Both A and R are true and R is the correct explanation of A.
Alkaline lysis exploits the structural differences between plasmid and genomic DNA. High pH causes both to denature, but circular plasmids remain interlinked. Upon neutralization, plasmids renature rapidly, while large, linear genomic DNA strands aggregate with proteins and are precipitated. This selective precipitation allows for the effective isolation of pure plasmid DNA from the bacterial cell lysates.

**11.** (A) Oligo(dT) cellulose affinity chromatography
Eukaryotic messenger RNA molecules are uniquely characterized by the presence of a poly-adenylated tail at their 3' terminus. Affinity chromatography utilizing cellulose beads conjugated with short thymidine oligomers allows for the specific capture of these transcripts. Under high-salt conditions, the poly-A tails hybridize to the oligo(dT), while other RNA types are washed away, enabling the purification of mRNA.

**12.** (C) To selectively precipitate denatured genomic DNA and proteins
During the alkaline lysis procedure, adding an acidic potassium acetate solution serves to neutralize the high pH of the mixture. This rapid neutralization causes the large, denatured genomic DNA and cellular proteins to form an insoluble complex with potassium dodecyl sulfate. The resulting white precipitate is easily removed by centrifugation, leaving the smaller, renatured plasmid DNA in the supernatant.

**13.** (D) $T_4$ DNA ligase
Genetic engineering relies on enzymes to join DNA fragments covalently. $T_4$ DNA ligase catalyzes the synthesis of a phosphodiester bond between a 3'-hydroxyl group and a 5'-phosphate group at the ends of double-stranded DNA. This reaction requires adenosine triphosphate as a cofactor to activate the enzyme, facilitating the repair of nicks or the ligation of sticky and blunt ends.

**14.** (C) It removes phosphate groups from the 5' ends to prevent selF-ligation
In molecular cloning, the vector DNA is often treated with alkaline phosphatase to remove the 5' terminal phosphate groups. This modification prevents the vector from recircularizing without an insert during the ligation step, as DNA ligase requires a phosphate for bond formation. Consequently, this treatment significantly increases the frequency of recombinant clones by ensuring that only insert-vector ligations occur.

**15.** (A) Treatment with alkaline phosphatase → Forward reaction with Polynucleotide Kinase → Purification
Radiolabeling the 5' end of a DNA fragment requires an available hydroxyl group. If the DNA is naturally phosphorylated, alkaline phosphatase must first be used to remove the existing phosphate. Subsequently, $T_4$ polynucleotide kinase catalyzes the transfer of a radioactive gamma-phosphate from ATP to the 3'-OH group. The process is finalized by purifying the labeled DNA from unincorporated nucleotides.

**16.** (D) Does not require a template strand
Terminal deoxynucleotidyl transferase is a specialized DNA polymerase that functions independently of a template strand. It catalyzes the repetitive addition of deoxynucleotides to the 3'-hydroxyl terminus of a DNA molecule. This unique property makes the enzyme indispensable for homopolymer tailing and for adding specific nucleotide sequences to the ends of DNA fragments in various molecular biology applications.

**17.** (A) Klenow fragment
The Klenow fragment is the large subunit of DNA polymerase I that retains polymerase and 3' to 5' exonuclease activities. When DNA fragments possess 5' protruding overhangs, the enzyme utilizes its polymerase activity to fill in the recessed 3' ends with complementary nucleotides. This reaction creates stable, blunt-ended fragments that are suitable for various cloning and ligation strategies.

**18.** (C) It operates optimally in the absence of magnesium ions
$T_4$ polynucleotide kinase is an essential enzyme that catalyzes the transfer of the gamma-phosphate from ATP to the 5'-hydroxyl terminus of nucleic acids. Like many other DNA-

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# ANSWERS

**1.** (C) 280 nm
Pure DNA preparations exhibit peak absorbance at 260 nm, while proteins absorb light most strongly at 280 nm due to aromatic amino acids like tyrosine and tryptophan. Measuring the ratio of absorbance at these two specific wavelengths allows researchers to quantify protein contamination. A lower $\frac{260}{280}$ ratio typically indicates a significant presence of residual proteins in the sample.

**2.** (C) Unstacking of nitrogenous bases
The hyperchromic effect refers to the significant increase in ultraviolet light absorbance observed when double-stranded DNA denatures into single strands. This phenomenon occurs because the nitrogenous bases are no longer stacked closely together in the helical structure. As the bases unstack and become more exposed, they absorb significantly more radiation at the 260 nm wavelength.

**3.** (A) Total length of unique DNA sequences
In DNA renaturation kinetics, sequence complexity represents the total length of the unique, non-repeating DNA sequences present in a genome. During a Cot curve analysis, DNA with higher complexity takes more time to find complementary strands and re-anneal. This parameter is fundamental for estimating genome size and identifying the proportion of repetitive versus unique sequences in various organisms.

**4.** (D) Guanine-Cytosine content
The melting temperature of double-stranded DNA is directly influenced by its base composition. Guanine-cytosine base pairs are connected by three hydrogen bonds, making them more stable than adenine-thymine pairs, which share only two. Consequently, DNA molecules with higher guanine-cytosine content require more thermal energy to disrupt these bonds, resulting in a higher temperature for strand separation and denaturation.

**5.** (B) Nucleic acids
During organic DNA extraction using a phenol-chloroform mixture, the solution separates into distinct phases based on polarity and density. Highly polar molecules, such as DNA and RNA, remain dissolved in the upper aqueous phase. Meanwhile, non-polar lipids and denatured proteins aggregate at the interphase or migrate into the lower organic phase, allowing for the effective purification of the nucleic acids.

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**6.** (C) To prevent foaming
Organic DNA extraction protocols involving phenol and chloroform often include isoamyl alcohol as a critical stabilization agent. The primary function of this component is to prevent the formation of foam during the vigorous mixing or centrifugation stages. Additionally, it helps to stabilize the interface between the aqueous and organic phases, ensuring a cleaner separation of the purified nucleic acids.

**7.** (D) Isopropanol
Alcohol precipitation is a standard technique used to concentrate and desalt nucleic acids. Adding isopropanol or ethanol in the presence of monovalent cations reduces the dielectric constant of the solvent. This change causes the negatively charged DNA phosphate backbone to interact with cations, leading to the formation of an insoluble precipitate that can be recovered through high-speed centrifugation.

**8.** (A) It acts as a chelating agent for divalent cations
Ethylenediaminetetraacetic acid is a vital component in lysis buffers because it binds to divalent cations like magnesium and calcium. These ions are essential cofactors for the activity of DNases and other nucleases that degrade genetic material. By sequestering these cations, the chemical effectively inhibits enzymatic degradation, thereby protecting the integrity of the genomic DNA during the extraction process.

**9.** (B) It acts as a strong RNase inhibitor and chaotropic agent
RNA is extremely susceptible to degradation by ubiquitous ribonuclease enzymes. Guanidinium thiocyanate is utilized in isolation protocols because it acts as a powerful chaotropic agent that simultaneously denatures proteins and inhibits RNase activity. This ensures that the

property makes it the ideal choice for separating small DNA fragments, such as those produced in sequencing reactions or microsatellite analysis. It is capable of resolving nucleic acid molecules that differ in length by as little as a single nucleotide, which is essential for precise molecular mapping.

**28.** (C) $MgCl_2$
Magnesium chloride is an indispensable component of the PCR master mix because it provides divalent magnesium ions. These ions serve as essential cofactors for the catalytic activity of Taq DNA polymerase, facilitating the formation of phosphodiester bonds. The concentration of these ions must be carefully optimized, as too little prevents amplification while too much can lead to non-specific products or primer-dimers.

**29.** (D) Intercalation between stacked nitrogenous bases
Ethidium bromide is a fluorescent dye widely used to detect DNA in electrophoresis gels. It functions by intercalating its flat, planar ring structure between the stacked nitrogenous bases of the double helix. When exposed to ultraviolet radiation, the DNA-bound dye absorbs energy and re-emits it as bright orange-red fluorescence, allowing for the sensitive visualization and quantification of the separated DNA fragments.

**30.** (B) I and III
Reverse transcriptase is an enzyme that synthesizes complementary DNA using an RNA template. Like other DNA polymerases, it requires a short primer with a free 3'-hydroxyl group to initiate the synthesis process. However, it notably lacks the 3' to 5' proofreading exonuclease activity found in many DNA-dependent polymerases, which results in a higher error rate during the transcription of RNA into DNA.

**31.** (B) It degrades the RNA strand of an RNA-DNA hybrid
In the synthesis of double-stranded cDNA, RNase H plays a specialized role by targeting the RNA strand within an RNA-DNA hybrid. It produces nicks and gaps in the RNA template, leaving behind small fragments that act as primers for DNA polymerase I. This process facilitates the synthesis of the second DNA strand, effectively replacing the original RNA with a complementary DNA sequence.

**32.** (D) RACE (Rapid Amplification of cDNA Ends)
Rapid Amplification of cDNA Ends is a specialized PCR-based technique used to obtain the full-length sequence of an RNA transcript. It is particularly effective for isolating the unknown 5' or 3' sequences that are often missing from standard cDNA libraries. By using gene-specific primers and specialized adaptors, researchers can amplify and characterize the extreme ends of the mRNA molecules precisely.

**33.** (A) Possesses higher thermal stability
Avian myeloblastosis virus reverse transcriptase is frequently selected for cDNA synthesis due to its superior thermal stability compared to the murine version. This property allows the reaction to be conducted at higher temperatures, which is beneficial for melting complex secondary structures in the RNA template. Consequently, it facilitates the synthesis of longer, full-length cDNA molecules from difficult or highly structured mRNA templates.

**34.** (C) $S_1$ nuclease
The classical hairpin method for cDNA synthesis involves the formation of a natural hairpin loop at the 3' end of the first strand, which acts as a primer. After the second strand is synthesized, the two DNA strands remain covalently linked at one end. $S_1$ nuclease, which specifically targets single-stranded DNA, is used to cleave the loop, resulting in a linear double-stranded molecule.

**35.** (A) Independent replication within the host cell
An origin of replication is a mandatory feature of any plasmid vector intended for molecular cloning. This specific DNA sequence is recognized by the host cell's replication machinery, allowing the plasmid to replicate independently of the bacterial chromosome. Without a functional origin, the plasmid would not be maintained through successive cell divisions, leading to the loss of the cloned gene in the population.

**36.** (D) The lacZ alpha peptide coding sequence
The pUC series of vectors facilitates the identification of recombinant clones through alpha-complementation. These vectors carry a short sequence encoding the alpha peptide of the beta-galactosidase enzyme. When transformed into a host expressing the complementary omega fragment, functional enzyme is formed, producing blue colonies on X-gal media. Insertion

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modifying enzymes, it strictly requires divalent cations, specifically magnesium ions, to function as a cofactor. The statement claiming it works optimally without magnesium is incorrect, as the metal ion is critical for catalytic efficiency.

**19.** (A) Cleave DNA at specific sites within or adjacent to their recognition sequence
Type II restriction endonucleases are the preferred tools for recombinant DNA technology due to their high specificity. Unlike other types, these enzymes recognize precise palindromic sequences and cleave the DNA at predictable, fixed positions within or very near those sites. This predictability allows researchers to generate specific DNA fragments with known terminal sequences, facilitating easy mapping and reliable molecular cloning.

**20.** (A) P-2, Q-3, R-4, S-1
Restriction endonucleases are classified based on their subunit composition and cleavage behavior. Type I enzymes cleave randomly far from their recognition site, while Type II enzymes cut precisely at or near specific sequences. Type III enzymes cut at a fixed distance approximately 25 base pairs away, and Type IV enzymes specifically target methylated DNA sequences, providing diverse tools for genome manipulation.

**21.** (B) Neoschizomers
Restriction enzymes that recognize the same target DNA sequence but cleave the strands at different positions are defined as neoschizomers. While they target identical nucleotides, the resulting fragments may have different types of ends, such as blunt versus protruding. This distinction is important in cloning strategies, as it determines whether the resulting DNA ends can be directly ligated to specific vectors.

**22.** (C) 5' protruding ends
The restriction enzyme BamHI recognizes the specific palindromic sequence 5'-GGATCC-3' and cleaves between the two guanine residues. This staggered cut results in the formation of 4-nucleotide long 5' protruding cohesive ends, often called sticky ends. These overhangs are highly useful in molecular cloning because they can easily hybridize with complementary sticky ends generated by the same or compatible enzymes.

**23.** (D) Relaxation of sequence specificity under suboptimal conditions
Star activity refers to the phenomenon where a restriction endonuclease loses its high sequence specificity and begins cleaving DNA at sites that differ slightly from its canonical recognition sequence. This behavior typically occurs under suboptimal laboratory conditions, such as high glycerol concentration, non-optimal buffer pH, or low ionic strength. Controlling these variables is essential to prevent unintended fragmentation of DNA.

**24.** (A) Inclusion of magnesium ions at recommended levels
To maintain the high fidelity of restriction enzyme digestion, optimal reaction conditions must be met. Including magnesium ions at the manufacturer's recommended concentrations provides the necessary cofactor for precise cleavage without inducing relaxed specificity. In contrast, factors like high glycerol content, high enzyme concentration, or incorrect pH levels frequently trigger star activity, leading to non-specific DNA degradation in the sample.

**25.** (B) II, III, I, IV
Agarose gel electrophoresis follows a specific chronological sequence. First, the agarose gel is cast, usually containing a DNA-binding dye for later visualization. Next, the DNA samples, mixed with a dense tracking dye, are loaded into the wells. An electric field is then applied to separate fragments by size. Finally, the resulting bands are visualized under ultraviolet light to analyze the migration.

**26.** (B) Melting temperature of the primers
The annealing temperature in a polymerase chain reaction is a critical parameter that ensures specific primer binding to the template DNA. It is typically calculated based on the melting temperature of the primer pair, usually set three to five degrees below the lower $T_m$. Setting the temperature correctly prevents non-specific amplification while allowing efficient hybridization and subsequent extension by the DNA polymerase.

**27.** (A) Resolving small DNA fragments with single-base differences
Polyacrylamide gel electrophoresis offers significantly higher resolution compared to agarose gels due to its smaller pore size. This

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(D) They transcribe the inserted genes

2004

**295.** Which methodology allows the identification of a specific gene clone using an antibody against the expressed protein?
(A) Immunological screening (e.g., Western blot/ colony immunoblot)
(B) Southern screening
(C) Northern screening
(D) Alpha-complementation

2013

**296.** The CRISPR-Cas9 system has revolutionized reverse genetics because it allows for:
(A) Highly specific, programmable, and efficient targeted DNA double-strand breaks
(B) Random genome-wide mutagenesis
(C) Direct synthesis of proteins without ribosomes
(D) Replacement of Dicer in RNAi

2017

**297.** Small interfering RNAs (siRNAs) direct the cleavage of target mRNAs. Where does this cleavage typically occur?
(A) Precisely in the middle of the region paired with the siRNA guide strand
(B) At the poly-A tail
(C) At the 5' cap
(D) Randomly throughout the transcript

2013

**298.** Which feature of filamentous bacteriophages (like $M_{13}$) makes them uniquely suitable for phage display?
(A) Their capsids can accommodate variable lengths of single-stranded DNA and surface fusion proteins
(B) They lyse the host cell rapidly
(C) They possess a lipid envelope
(D) They only infect eukaryotic cells

2004

**299.** In the context of genetically modified organisms, a "reporter gene" like GUS is particularly advantageous because:
(A) It allows for the visual tracking of gene expression spatial patterns in tissues
(B) It confers antibiotic resistance
(C) It kills non-transgenic cells
(D) It acts as a viral origin of replication

2019

**300.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** The primary ethical argument against patenting human genes is that genes are naturally occurring discoveries.
**Reason (R):** Naturally occurring genes are not human inventions, and patents on them may hinder medical research.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

*********

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of DNA into the multiple cloning site disrupts this sequence, resulting in white colonies.

**37.** (B) Can accommodate significantly larger DNA inserts
Bacteriophage lambda vectors are highly efficient tools for constructing genomic libraries because they can carry much larger DNA inserts than standard plasmids. While plasmids are generally limited to small fragments, lambda replacement vectors can accommodate inserts between 10 and 25 kilobases. Furthermore, the in vitro packaging process ensures that only recombinant molecules of the appropriate size are successfully introduced into the host bacteria.

**38.** (C) Plasmids and $M_{13}$ bacteriophages
Phagemids are sophisticated hybrid vectors that incorporate the origin of replication from both a plasmid and a filamentous bacteriophage like $M_{13}$. This dual nature allows them to be propagated as double-stranded circular plasmids under normal conditions. However, when the host is infected with a helper phage, the vector can be packaged and secreted as single-stranded DNA, facilitating sequencing and site-directed mutagenesis.

**39.** (D) Single-stranded DNA for sequencing and mutagenesis
$M_{13}$ bacteriophage vectors are uniquely suited for applications requiring single-stranded DNA. Because the phage lifecycle involves the production and secretion of single-stranded genomic copies, researchers can easily harvest high-purity single-stranded templates from the culture medium. This capability is essential for classical Sanger sequencing methods and for various site-directed mutagenesis protocols that require the hybridization of synthetic oligonucleotides to a single-stranded template.

**40.** (C) III
Cosmids are specialized hybrid vectors containing the cos site of bacteriophage lambda, allowing them to be packaged in vitro into phage heads. They can carry large DNA inserts up to 45 kilobases. Importantly, once inside the bacterial cell, cosmids circularize and replicate as large plasmids rather than entering a lytic cycle, meaning they do not naturally lyse the host cell.

**41.** (C) YAC (Yeast Artificial Chromosome)
For projects involving the mapping of complex eukaryotic genomes, yeast artificial chromosomes offer the highest carrying capacity among all common vector systems. They can accommodate DNA fragments ranging from several hundred kilobases to over one megabase. This large capacity allows researchers to represent entire gene clusters or large genomic regions in a single clone, significantly reducing the total number of clones required.

**42.** (C) $F^-$plasmid of E. coli
Bacterial artificial chromosomes are based on the naturally occurring $F^-$plasmid of Escherichia coli. This plasmid is highly stable because it replicates at a very low copy number, typically one to two copies per cell. This feature is advantageous for cloning large genomic fragments, as it minimizes the risk of DNA rearrangements or deletions that can occur in high-copy number plasmid systems.

**43.** (A) Telomeric sequences
Yeast artificial chromosomes are designed to function like natural chromosomes within a yeast cell. To maintain stability as linear molecules, they must contain telomeric sequences at both ends, along with a centromere for segregation and an origin of replication. While bacterial artificial chromosomes also require an origin and selectable markers, they exist as circular molecules and do not utilize telomeres for genomic stability.

**44.** (D) Avoid random integration and position effects
Mammalian artificial chromosomes are engineered to exist as independent, non-integrating genetic elements within a human cell. By remaining episomal, they avoid the risks associated with random integration into the host genome, such as the disruption of endogenous genes or variable expression due to position effects. This stability and safety make them highly promising tools for long-term gene therapy applications in human patients.

**45.** (A) I, II, III and V
Retroviral vectors are powerful tools for gene delivery because they integrate their genetic material directly into the host genome, ensuring stable expression. They package their genome as single-stranded RNA and utilize reverse transcriptase to form DNA once inside the cell. Most retroviruses require dividing cells for

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that antibiotic. This phenotypic change allows researchers to distinguish between recombinant clones and those containing the original, non-recombinant plasmid.

**64.** (C) Beta-galactosidase
In blue-white screening, the enzyme beta-galactosidase catalyzes the cleavage of the colorless substrate X-gal into a galactose molecule and an indigo-colored compound. This activity occurs when the alpha peptide from the vector successfully complements the omega fragment in the host cell. The resulting blue color in the colonies indicates the presence of a functional enzyme and, conversely, a non-recombinant plasmid vector.

**65.** (A) III, IV, II, I
Colony hybridization is a multi-step process for screening libraries. First, bacterial colonies are transferred to a nitrocellulose membrane. The cells are then lysed, and the DNA is denatured and fixed to the membrane. Next, the membrane is hybridized with a labeled nucleic acid probe. Finally, autoradiography or a similar detection method is used to identify the specific colonies containing the DNA sequence of interest.

**66.** (A) Sodium bisulfite
Sodium bisulfite is a chemical agent used in molecular biology for in vitro mutagenesis. It specifically targets single-stranded DNA and catalyzes the deamination of cytosine residues, converting them into uracil. Upon subsequent DNA replication, the uracil is paired with adenine, resulting in a permanent C-to-T transition mutation. this specific chemical property makes it a valuable tool for studying gene function and DNA methylation patterns.

**67.** (B) DpnI
The QuikChange site-directed mutagenesis protocol uses PCR to generate mutant DNA strands. The original parental DNA template, derived from a bacterial source, is methylated. DpnI is a restriction enzyme that specifically cleaves only methylated DNA sequences. By treating the PCR product with DpnI, the non-mutated parental template is selectively degraded, leaving behind the newly synthesized, unmethylated mutant DNA for transformation into host cells.

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**68.** (A) Relies on random mutagenesis and screening for desired traits
Directed evolution is a powerful protein engineering strategy that mimics the process of natural selection in the laboratory. It involves generating a library of variants through random mutagenesis and then screening or selecting those that exhibit improved or novel functions. Unlike rational design, it does not require detailed knowledge of the protein's structure or mechanism, making it highly effective for optimizing complex traits.

**69.** (B) Replacing a restriction fragment with a mutant segment
Cassette mutagenesis is a targeted method for introducing specific mutations into a DNA sequence. It involves removing a small segment of the gene using two restriction enzymes and then replacing it with a synthetic double-stranded oligonucleotide "cassette" that contains the desired sequence changes. This approach allows for the efficient and precise modification of a specific region within a larger DNA molecule or vector.

**70.** (C) II and III
Reverse genetics is an approach used to determine the biological function of a gene by starting with its DNA sequence and then analyzing the resulting phenotype. Techniques such as gene knockout, knockdown, or site-directed mutagenesis are employed to disrupt or alter the gene's function. By observing the changes in the organism's traits, researchers can infer the natural role of the specific gene being studied.

**71.** (A) Embryonic stem (ES) cells
Creating a knockout mouse involves the precise replacement of an endogenous gene with a non-functional version via homologous recombination. This genetic modification is typically performed in embryonic stem cells because they are pluripotent and can be cultured in vitro. After successful selection, these modified ES cells are injected into blastocysts, where they contribute to the germline of the resulting chimeric mice.

**72.** (B) Conditional or tissue-specific gene knockouts
The Cre-loxP system is a sophisticated tool for site-specific recombination in mammalian genomes. It utilizes the Cre recombinase enzyme, which recognizes and cleaves DNA at specific 34-base pair sequences called loxP sites. By flanking

end of the 16S ribosomal RNA. This interaction correctly positions the small ribosomal subunit to initiate translation at the AUG start codon, ensuring the efficient production of the encoded protein in bacteria.

**55.** (B) Promoter → Ribosome binding site → Start codon → Multiple cloning site → Terminator
A standard prokaryotic expression vector organizes functional elements in a precise 5' to 3' order. The promoter initiates transcription, followed by the ribosome binding site for translation initiation. The start codon marks the beginning of the protein sequence, leading into the multiple cloning site where the gene is inserted. Finally, a transcriptional terminator ensures the mRNA synthesis ends at the correct position.

**56.** (C) Polyhistidine (His) tag
The polyhistidine tag is a widely used affinity tag consisting of six to ten consecutive histidine residues fused to a recombinant protein. This tag has a high affinity for divalent metal ions, such as nickel or cobalt, immobilized on a resin. This interaction allows for the efficient purification of the tagged protein from complex cellular lysates in a single chromatographic step using imidazole for elution.

**57.** (B) Linkers
Linkers are short, synthetic, double-stranded DNA molecules that contain one or more restriction enzyme recognition sites. They are typically ligated to the blunt ends of a DNA fragment using $T_4$ DNA ligase. Subsequent digestion with the appropriate restriction enzyme creates cohesive sticky ends, allowing the fragment to be easily inserted into a vector that has been cut with the same enzyme.

**58.** (B) Already possess preformed cohesive (sticky) ends
Adaptors are similar to linkers but differ in that they are synthesized with preformed cohesive ends on one side. This design eliminates the need for restriction enzyme digestion after ligation to the blunt-ended DNA fragment. To prevent selF-ligation, the sticky ends of the adaptors are often dephosphorylated, ensuring that the fragments can only be joined to the vector once the ends are reactivated.

**59.** (B) Terminal deoxynucleotidyl transferase
Homopolymer tailing is a technique used to join DNA fragments by adding a string of identical nucleotides, such as poly-A or poly-T, to their 3' ends. This reaction is catalyzed by terminal deoxynucleotidyl transferase, a template-independent polymerase. When two fragments with complementary homopolymer tails are mixed, they hybridize, allowing for the stable ligation of the molecules even if they lack natural restriction sites.

**60.** (A) P-3, Q-4, R-1, S-2
Different gene delivery methods utilize distinct mechanisms to transport DNA into cells. Electroporation uses electrical pulses to create pores, while lipofection involves encapsulating DNA in lipid vesicles. Microinjection is a mechanical process using a fine needle for direct insertion, and calcium phosphate transfection relies on the co-precipitation of DNA to facilitate uptake by endocytosis, providing various options for different cell types.

**61.** (B) Ice-cold calcium chloride solution
Chemical competence in bacteria is achieved by treating the cells with a cold solution of divalent cations, typically calcium chloride. This treatment alters the cell wall and membrane properties, facilitating the binding of DNA to the cell surface. A subsequent brief heat shock creates a thermal gradient that draws the DNA into the cytoplasm, allowing for the successful transformation of the bacterial host.

**62.** (C) Microinjection
Microinjection is a highly precise and direct method for delivering genetic material into animal cells. Using a specialized micromanipulator and a glass capillary needle, the DNA is physically injected directly into the nucleus or cytoplasm. While labor-intensive and low-throughput, this technique guarantees that the DNA bypasses cellular barriers, making it particularly useful for generating transgenic animals from fertilized oocytes or embryonic stem cells.

**63.** (D) Insertional inactivation
Insertional inactivation occurs when a foreign DNA fragment is ligated into a restriction site located within a functional gene on a vector. In the case of pBR322, inserting DNA into the tetracycline resistance gene disrupts its sequence, rendering the host cell sensitive to

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nuclear entry, although lentiviruses have the unique ability to transduce non-dividing cells through the nuclear pores.

**46.** (D) Adenovirus
Adenoviral vectors are widely used for gene delivery due to their ability to transduce a broad range of cell types, including both dividing and quiescent cells. They possess a large double-stranded DNA genome and typically remain episomal within the nucleus rather than integrating. This leads to high-level, transient expression of the delivered gene, making them suitable for vaccines and short-term therapeutic applications.

**47.** (A) Host cell cytoplasm
Vaccinia virus is a large, complex poxvirus that is unique among DNA viruses because its entire replication cycle occurs within the cytoplasm of the host cell. The virus carries its own transcription machinery, including a DNA-dependent RNA polymerase, allowing it to function independently of the host cell nucleus. This characteristic makes vaccinia vectors highly efficient for the high-level expression of recombinant proteins.

**48.** (A) Spodoptera frugiperda ($Sf_9$) insect cells
The baculovirus expression vector system is a popular choice for producing complex eukaryotic proteins. In this system, recombinant baculoviruses are used to infect insect cell lines, most commonly derived from the fall armyworm. These host cells provide a eukaryotic environment that facilitates proper protein folding and complex post-translational modifications, such as glycosylation, resulting in functional proteins that resemble their natural human counterparts.

**49.** (B) Replicate in two different host species
Shuttle vectors are versatile genetic tools engineered to replicate in two distinct host organisms, typically a prokaryote like E. coli and a eukaryote like yeast or mammalian cells. They contain two different origins of replication and two sets of selectable markers tailored for each host. This allows researchers to perform initial cloning and manipulation in bacteria before transferring the vector for expression studies.

**50.** (A) Both A and R are true and R is the correct explanation of A.
The methylotrophic yeast Pichia pastoris is a superior host for industrial protein production compared to traditional brewer's yeast. It utilizes the exceptionally strong and tightly regulated AOX1 promoter, which is induced by methanol. Furthermore, Pichia pastoris secretes very few endogenous proteins into the culture medium, which significantly simplifies the purification process for the secreted recombinant protein, enhancing overall efficiency and yield.

**51.** (B) Saccharomyces cerevisiae
The 2-micron circle is a naturally occurring, high-copy-number plasmid found within the nucleus of the yeast Saccharomyces cerevisiae. It is approximately 6,300 base pairs long and replicates independently of the host chromosomes. This plasmid has been extensively modified to create various yeast cloning and expression vectors, providing a reliable means for the stable maintenance and high-level expression of foreign genes in yeast.

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**52.** (C) Centromeric sequence (CEN)
For a yeast vector to be maintained stably through successive rounds of cell division, it must be segregated accurately between mother and daughter cells. While autonomously replicating sequences allow the vector to replicate, the addition of a centromeric sequence ensures that the plasmid associates with the spindle apparatus during mitosis. This precise segregation prevents the rapid loss of the plasmid from the yeast population.

**53.** (B) Toxicity to the host cell during early growth
Inducible promoters are critical in expression vectors when the target recombinant protein is toxic or inhibitory to the host cell. By using an inducible system, researchers can grow the culture to a high density before triggering protein synthesis. This strategy prevents the early accumulation of the protein from hindering cell growth, thereby maximizing the total yield and stability of the expression system.

**54.** (B) Ribosome binding site for translation initiation
The Shine-Dalgarno sequence is a short, purine-rich region located upstream of the start codon in prokaryotic messenger RNA. It serves as the ribosome binding site by hybridizing with the 3'

a target gene with loxP sites, researchers can achieve conditional or tissue-specific gene deletion, allowing for the study of gene function in particular developmental stages or specific organ systems.

**73.** (B) DNA microarrays
DNA microarrays are powerful genomic tools that allow for the simultaneous monitoring of the expression levels of thousands of genes. They consist of a solid surface onto which thousands of specific DNA probes are attached in a precise grid. By hybridizing fluorescently labeled cDNA from a sample to these probes, researchers can quantify the activity of the entire transcriptome under different biological conditions.

**74.** (A) RNase III-like endonuclease
Dicer is a critical enzyme in the RNA interference pathway that processes long double-stranded RNA and precursor microRNA into small interfering RNAs or mature miRNAs. It is an RNase III-like endonuclease that cleaves the RNA into short fragments, typically twenty-one to twenty-three nucleotides in length. These small RNA molecules then serve as the guides for targeted gene silencing within the RISC complex.

**75.** (C) Argonaute (Ago)
The RNA-induced silencing complex is the central effector of RNA interference. Its key catalytic component is the Argonaute protein, which binds to the small interfering RNA guide strand. The protein uses this guide to identify complementary messenger RNA molecules. Once bound, the Argonaute protein acts as an endonuclease to cleave the target mRNA, effectively silencing the gene by preventing its translation into protein.

**76.** (A) Imperfect base pairing leading to translational repression
Endogenous microRNAs typically function by binding to the 3' untranslated regions of target messenger RNAs. Unlike siRNAs, which usually require perfect complementarity for cleavage, miRNAs often exhibit imperfect base pairing. This binding prevents the translation machinery from successfully producing the encoded protein, resulting in translational repression. This mechanism allows a single miRNA to regulate multiple target genes simultaneously within a cell.

**77.** (A) pre-miRNA
The biogenesis of microRNAs involves several processing steps. Initially, long primary transcripts are cleaved in the nucleus by the Drosha enzyme to form hairpin-shaped precursor miRNAs. These pre-miRNAs are then recognized by the Exportin-5 protein and transported through the nuclear pore complex into the cytoplasm. Once in the cytoplasm, the pre-miRNA is further processed by the Dicer enzyme into the mature, functional miRNA.

**78.** (A) They possess an uncharged pseudopeptide backbone lacking electrostatic repulsion
Peptide nucleic acids are synthetic DNA mimics where the sugar-phosphate backbone is replaced by an uncharged N-aminoethylglycine linkage. Because the backbone lacks the negative charge found in natural nucleic acids, there is no electrostatic repulsion when a PNA strand binds to complementary DNA or RNA. This results in extraordinarily high binding affinity and thermal stability, making them excellent tools for diagnostic and therapeutic applications.

**79.** (D) Recruiting DNA polymerase for replication block
Antisense oligonucleotides are short, single-stranded nucleic acids designed to bind to specific mRNA transcripts. They induce gene silencing by physically blocking ribosome binding or by recruiting RNase H to degrade the RNA-DNA hybrid. They can also be used to modulate splicing patterns. Importantly, they do not function by recruiting DNA polymerase, as their primary targets are RNA molecules rather than genomic DNA.

**80.** (B) II
Morpholinos are synthetic molecules where the riboside is replaced by a morpholine ring and the linkage is a non-ionic phosphorodiamidate. These modifications make them highly resistant to nuclease degradation and uncharged. Unlike traditional antisense oligonucleotides, morpholinos do not recruit RNase H. Instead, they function primarily as steric blocks, preventing translation or splicing by physically obstructing the binding of essential cellular machinery.

**81.** (A) A phage coat protein (e.g., pIII or pVIII)
Phage display is a laboratory technique for studying protein-protein interactions. A gene

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**116.** (A) The ampicillin and tetracycline resistance genes
The pBR322 plasmid was one of the first widely used cloning vectors. It was engineered to contain unique restriction sites specifically within its two antibiotic resistance genes. For example, the BamHI site is located within the tetracycline resistance gene. Inserting a DNA fragment at this site disrupts the gene's function, allowing for the selection of recombinant clones through the phenomenon of insertional inactivation.

**117.** (C) 38 - 52 kb
The in vitro packaging of bacteriophage lambda DNA is highly sensitive to the total length of the DNA molecule. The packaging machinery only accepts DNA that is between 78% and 105% of the wild-type genome size, which translates to a functional range of approximately 38 to 52 kilobases. This physical constraint serves as a built-in selection mechanism, ensuring that only recombinants with the correct size are viable.

**118.** (A) Origin of replication (f1 ori)
$M_{13}$ vectors are specialized cloning tools derived from a filamentous bacteriophage. They contain a specific origin of replication, known as the f1 ori, which directs the synthesis of single-stranded DNA. During the phage lifecycle, the double-stranded replicative form is converted into single-stranded circular DNA, which is then packaged into the phage particle and secreted from the host cell without causing lysis.

**119.** (A) Capacity for eukaryotic post-translational modifications like glycosylation
Using yeast as a host for recombinant protein production offers significant advantages over bacterial systems. As eukaryotes, yeast cells possess the necessary cellular machinery for complex post-translational modifications, including proper folding, disulfide bond formation, and glycosylation. This ensures that the recombinant human or animal proteins are biologically active and structurally similar to their naturally occurring counterparts, which is often not possible in E. coli.

**120.** (A) II and IV
Yeast artificial chromosomes are linear vectors that can carry massive DNA inserts, often exceeding one megabase. They are composed of essential yeast elements, including origins, centromeres, and telomeres. However, they are known to be relatively unstable and are prone to forming chimeric clones, where fragments from different parts of the genome are joined together. Furthermore, they cannot be propagated in E. coli without significant modification.

**121.** (A) Can transduce both dividing and non-dividing cells efficiently
Adenoviral vectors are highly efficient tools for gene therapy because they can deliver genetic material into a wide variety of cell types. Unlike some other viral systems, they do not require the host cell to be actively dividing to achieve successful transduction. Because the viral DNA typically remains as an independent episome in the nucleus, the gene expression is usually high but transient.

**122.** (A) Extremely strong and active in the very late phase of the infection cycle
In the baculovirus expression system, the polyhedrin promoter is utilized for the high-level production of recombinant proteins. This promoter is naturally one of the strongest known in nature and becomes highly active during the very late stage of the viral infection cycle. This allows for the massive accumulation of the target protein in insect cells, often reaching up to fifty percent of the total cellular protein.

**123.** (A) Prevent transcriptional read-through which could destabilize the plasmid
Transcriptional terminators are essential components of expression vectors, placed downstream of the cloned gene. Their primary function is to provide a signal for the RNA polymerase to stop transcription, preventing the enzyme from continuing into other regions of the plasmid. This prevents transcriptional read-through, which could otherwise interfere with plasmid replication or the expression of selectable markers, leading to overall plasmid instability.

**124.** (A) Secretion of the recombinant protein into the periplasm or culture medium
Signal peptides are short amino acid sequences at the N-terminus of a protein that direct its transport to specific cellular compartments. In recombinant expression, fusing a signal sequence to the target protein allows it to be secreted across the cell membrane into the periplasm or the surrounding culture medium. This secretion

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**90.** (A) Both A and R are true and R is the correct explanation of A.
Lipid nanoparticles are essential for the effective delivery of mRNA vaccines. The use of ionizable lipids is a key feature; these lipids remain neutral at a physiological pH to minimize toxicity in the blood. However, once internalized into acidic endosomes, they become protonated and positively charged. This change triggers the disruption of the endosomal membrane, allowing the mRNA to escape into the cytoplasm.

**91.** (A) Evade the reticuloendothelial system and increase circulation time
PEGylation involves the covalent attachment of polyethylene glycol chains to the surface of polymeric nanocarriers. This modification creates a hydrated layer that prevents the adsorption of serum proteins and recognition by the immune system. As a result, the nanocarriers can evade the reticuloendothelial system, leading to a significantly increased circulation time in the bloodstream and improving the overall efficacy of the delivered drug.

**92.** (C) Solid tumors
The enhanced permeability and retention effect is a unique characteristic of the vasculature in solid tumors. Rapidly growing tumors develop leaky, disorganized blood vessels with large gaps, and they often lack efficient lymphatic drainage. This environment allows nanocarriers to pass from the blood into the tumor tissue and remain there for extended periods, providing a passive targeting mechanism for the selective delivery of anticancer drugs.

**93.** (B) Agrobacterium tumefaciens
The tumor-inducing plasmid is a large plasmid found naturally in the soil bacterium Agrobacterium tumefaciens. This organism has the unique ability to transfer a specific segment of its DNA into the genome of a host plant, where it causes the formation of crown gall tumors. This natural horizontal gene transfer mechanism has been extensively modified by scientists to create a powerful tool for plant genetic engineering.

**94.** (D) 25 bp direct repeat border sequences
The transfer DNA region is the specific segment of the Ti plasmid that is integrated into the plant genome during infection. It is defined and delimited by two 25-base pair direct repeat sequences known as the left and right borders. These border sequences are the only essential cis-acting elements required for the transfer process, as the vir genes on the plasmid provide the necessary machinery.

**95.** (A) uidA (GUS)
The GUS gene, encoding the enzyme beta-glucuronidase, is a widely used scorable reporter gene in plant transgenesis. When the substrate X-gluc is added to transgenic tissues expressing this gene, the enzyme cleaves it to produce a blue precipitate. This allows researchers to easily visualize the spatial and temporal patterns of gene expression across different tissues and developmental stages in the modified plant.

**96.** (A) Selectable markers confer survival under toxic conditions, whereas scorable markers produce a visually identifiable phenotype.
Selectable and scorable markers serve different purposes in genetic engineering. Selectable markers, such as antibiotic resistance genes, allow for the survival of only those cells that have successfully taken up the DNA. In contrast, scorable markers, like green fluorescent protein or GUS, produce a visible signal that allows researchers to identify transgenic cells or tissues without killing those that are non-transgenic.

**97.** (A) The unique insertion of a specific recombinant DNA construct into a specific genomic location
In the context of biotechnology and regulation, a transgenic event refers to a single, unique instance of DNA integration into the host genome. Even if the same DNA construct is used, every successful transformation results in a different event because the insertion occurs at a different genomic location. Regulatory agencies evaluate each event separately for its safety, stability, and potential environmental impact before commercialization.

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**98.** (A) Cause severe or lethal disease primarily via aerosol transmission
Biosafety Level 3 containment is required for laboratory work involving indigenous or exotic agents that can cause serious or potentially lethal disease through respiratory transmission. These facilities feature specialized ventilation systems, including directional airflow and high-efficiency particulate air filtration. Personnel must follow strict protocols and wear protective equipment to prevent laboratory-acquired infections and

strand, called the passenger strand, is typically cleaved and degraded or simply discarded from the complex. Only the guide strand remains to direct the silencing of the complementary target transcript.

**134.** (A) Petunia hybrida
The phenomenon of post-transcriptional gene silencing was first observed in petunia plants. When researchers attempted to darken the flower color by introducing additional copies of a pigment-producing gene, they unexpectedly found that many of the transgenic plants had white or variegated flowers. This discovery, originally called cosuppression, provided the first evidence that exogenous DNA could trigger the degradation of both transgene and endogenous RNA.

**135.** (A) PNA lacks a sugar-phosphate backbone
Peptide nucleic acids are synthetic DNA analogs that differ significantly in their chemical structure from conventional oligonucleotides. While standard DNA and RNA possess a sugar-phosphate backbone, PNAs utilize a pseudopeptide backbone made of N-aminoethylglycine units. This structural change removes the negative charge associated with phosphates, leading to stronger binding with natural nucleic acids and increased resistance to degradation by nucleases and proteases.

**136.** (A) A phenotype (the displayed protein) to its genotype (the encapsulated DNA)
Phage display technology is unique because it physically links the protein expressed on the surface of a bacteriophage to the genetic information contained within the phage particle. This direct connection between phenotype and genotype allows researchers to screen vast libraries of protein variants for specific binding properties. Once a successful binder is identified, the corresponding DNA sequence can be easily recovered and sequenced.

**137.** (A) Grafting the mouse complementarity determining regions (CDRs) onto a human antibody framework
Humanizing a mouse monoclonal antibody involves a sophisticated engineering process to make it more compatible with the human immune system. The most common strategy is CDR grafting, where the antigen-binding loops of the mouse antibody are transplanted onto a human antibody framework. This results in a molecule that is mostly human but retains the original mouse-derived specificity, significantly reducing the risk of an adverse immune reaction.

**138.** (A) Methionine
Early versions of recombinant human growth hormone produced in E. coli contained an additional methionine residue at the amino terminus. This occurred because the bacterial translation machinery initiates protein synthesis with formyl-methionine, and the host cells were not always able to remove it from the large protein. Modern production methods have been refined to ensure the N-terminal methionine is removed, resulting in a protein identical to natural human somatotropin.

**139.** (A) Saccharomyces cerevisiae
The recombinant subunit vaccine for Hepatitis B is produced by expressing the viral surface antigen in yeast cells, specifically Saccharomyces cerevisiae. The yeast cells are engineered to produce large quantities of the HBsAg protein, which naturally assembles into virus-like particles. These particles are then purified and used as the primary component of the vaccine, providing a safe and effective means to elicit protective immunity.

**140.** (A) P-4, Q-2, R-1, S-3
Microbial strain improvement utilizes diverse strategies to optimize industrial production. Rational design applies targeted modifications based on metabolic knowledge, while protoplast fusion combines entire genomes by merging cells. Mutagenesis involves random genetic changes using physical or chemical agents. Finally, directed evolution mimics natural selection through iterative rounds of mutation and screening, allowing researchers to evolve strains with highly specialized and improved industrial characteristics.

**141.** (A) Reroute carbon fluxes to maximize the yield of a desired product
The primary objective of metabolic engineering is to optimize the internal pathways of a microorganism to enhance the production of a target metabolite. This often involves rerouting carbon fluxes away from biomass production or competing side reactions toward the desired biosynthetic pathway. By manipulating the regulatory and enzymatic networks of the

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applied, the initial breaking of a few base pairs destabilizes the surrounding structure, causing the remaining strands to separate rapidly over a narrow temperature range. This results in a characteristic sigmoidal melting curve. The temperature at the midpoint of this transition is defined as the melting temperature for the molecule.

**108.** (A) The difference in supercoiling, which restricts ethidium bromide intercalation in plasmids
Density gradient centrifugation with cesium chloride and ethidium bromide separates plasmids from genomic DNA based on their structural differences. Plasmids are covalently closed circular molecules that are highly supercoiled, which limits the amount of ethidium bromide that can intercalate into the DNA. Because intercalation reduces the density of DNA, the less-intercalated plasmids remain denser and form a distinct band lower in the gradient.

**109.** (A) Cohesive (sticky) ends but can also ligate blunt ends
$T_4$ DNA ligase is a versatile enzyme used in genetic engineering to join DNA fragments. While it is most efficient at ligating cohesive ends, where complementary overhangs facilitate the stable alignment of the strands, it is also capable of ligating blunt-ended fragments. However, blunt-end ligation generally requires higher concentrations of both the enzyme and the DNA to overcome the lack of base-pairing stability.

**110.** (B) I and III
Type III restriction enzymes are unique complexes that possess both restriction and modification activities. They require adenosine triphosphate and S-adenosylmethionine for their enzymatic function. Unlike Type II enzymes, they cleave the DNA at a specific distance away from their recognition site, usually about 25 base pairs. Their multi-functional nature distinguishes them from the simpler Type II enzymes more commonly used in molecular cloning.

**111.** (A) $T_4$ DNA polymerase (exonuclease activity)
$T_4$ DNA polymerase is a multifunctional enzyme that possesses strong 3' to 5' exonuclease activity. When DNA fragments have protruding 3' overhangs, this exonuclease activity allows the enzyme to digest the single-stranded tail until it reaches the double-stranded region. If deoxynucleotides are present, the enzyme's polymerase activity can then stabilize the end, effectively converting a 3' protruding terminus into a blunt end.

**112.** (A) It lacks 3' to 5' proofreading exonuclease activity
Taq DNA polymerase is a thermostable enzyme widely used in PCR, but it lacks a 3' to 5' proofreading exonuclease activity. This deficiency means the enzyme cannot correct base-incorporation errors during synthesis, leading to a higher mutation rate. For amplifying large or complex genomic templates where sequence fidelity is paramount, other polymerases with proofreading capabilities or specialized enzyme mixtures are typically preferred.

**113.** (A) Dideoxynucleoside triphosphates (ddNTPs)
The Sanger sequencing method relies on the principle of chain termination to determine DNA sequences. The reaction includes small amounts of dideoxynucleoside triphosphates, which lack the 3'-hydroxyl group necessary for the formation of a phosphodiester bond. When a ddNTP is incorporated into the growing DNA strand, elongation is permanently halted, resulting in a series of fragments that reveal the sequence of the template.

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**114.** (A) Generates RNA fragments that serve as primers for the second strand synthesis
During the construction of a cDNA library, the RNase H activity of reverse transcriptase is essential for synthesizing the second DNA strand. The enzyme partially degrades the original RNA template, leaving behind small RNA fragments hybridized to the first cDNA strand. These residual RNA pieces serve as natural primers for DNA polymerase I, allowing for the synthesis of the complementary second DNA strand.

**115.** (A) Represents only the genes that are actively transcribed in the source cells
A cDNA library is fundamentally different from a genomic library because it is derived from messenger RNA. Consequently, it only contains sequences that were actively transcribed in the tissue or cell type at the time of isolation. These libraries lack introns, promoters, and other non-coding regulatory elements, making them particularly useful for studying protein-coding sequences and gene expression patterns.

**150.** (A) Both A and R are true and R is the correct explanation of A.
Legal and ethical standards generally prohibit the patenting of naturally occurring, unmodified human genes. These sequences are considered products of nature rather than human inventions. Because discovering a gene does not involve an inventive step, granting patents could hinder medical research and restrict access to genetic testing. Therefore, jurisdictions like the United States have ruled that isolated natural DNA sequences are not patentable subject matter.

**151.** (A) DNA sequence complexity
The rate at which denatured DNA strands re-anneal is primarily determined by their sequence complexity. In simple genomes with many repetitive sequences, complementary strands find each other quickly. In contrast, complex genomes with many unique sequences require significantly more time for proper hybridization. This relationship, analyzed through Cot curves, is a fundamental tool for estimating the size and organization of diverse genomic DNA samples.

**152.** (A) Silica-based spin columns and chaotropic salts
After resolving DNA fragments by agarose gel electrophoresis, the specific bands can be recovered through a process called gel extraction. This typically involves melting the gel slice and using silica-based spin columns in the presence of high concentrations of chaotropic salts. The DNA binds to the silica while the agarose and buffer are washed away, allowing the purified DNA to be eluted for further cloning or analysis.

**153.** (A) Fewer clones to cover the entire genome
Bacterial artificial chromosomes are favored for genome sequencing because they can carry much larger DNA inserts, typically between 100 and 300 kilobases, compared to cosmids. This larger capacity means that a significantly smaller number of clones is required to achieve full coverage of the genome. Additionally, BACs are highly stable in the host, making them reliable tools for large-scale physical mapping and sequencing projects.

**154.** (A) Formation of aqueous pores in the lipid bilayer via electrical pulses
Electroporation is a highly effective physical method for gene delivery. It involves applying a high-voltage electrical pulse to a cell suspension, which transiently destabilizes the cell membrane. This pulse causes the temporary formation of small aqueous pores in the lipid bilayer, allowing exogenous DNA or other molecules to enter the cytoplasm. Once the pulse ends, the pores naturally seal, leaving the genetic material inside.

**155.** (A) A lentiviral vector
RNA interference is often used to achieve long-term gene silencing in mammalian cells. While standard siRNAs provide only transient effects, delivering short hairpin RNA via a lentiviral vector allows for the stable integration of the silencing construct into the host genome. This ensures that the shRNA is continuously expressed and passed on to daughter cells, providing permanent knockdown of the target gene even in non-dividing populations.

**156.** (A) Dense, insoluble aggregates of misfolded recombinant proteins in bacteria
When high levels of recombinant proteins are expressed in bacteria like E. coli, they often fail to fold correctly, leading to the formation of inclusion bodies. These are dense, insoluble aggregates of misfolded protein that can be seen under a microscope. While inclusion bodies protect the protein from degradation, they require harsh chemical treatments and complex refolding procedures to recover the biologically active, functional protein.

**157.** (A) DNA polymerase I
DNA polymerases, including DNA polymerase I, are unable to initiate the synthesis of a new DNA strand from scratch. They strictly require a pre-existing primer, which can be either RNA or DNA, that provides a free 3'-hydroxyl group. The enzyme then catalyzes the addition of deoxynucleotides by forming phosphodiester bonds, extending the new strand in the 5' to 3' direction based on the template.

**158.** (A) They cleave at random distances far from their recognition site
Type I restriction endonucleases are complex, multi-functional enzymes that are not commonly used in molecular cloning. Although they recognize specific DNA sequences, they cleave the DNA at random locations that can be several thousand base pairs away from the recognition site. This lack of predictability makes it impossible to generate specific, reproducible

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facilitates proper folding and greatly simplifies the subsequent purification of the protein.

**125.** (A) I, II, III and V
Adaptors are short, double-stranded synthetic oligonucleotides used to add specific restriction sites to blunt-ended DNA fragments. They are designed with one blunt end for ligation to the target DNA and one preformed cohesive overhang. To prevent unwanted selF-ligation of the adaptors, the cohesive ends are typically dephosphorylated. This ensures that the fragments can only be joined to the vector after proper enzymatic treatment.

**126.** (A) dCTP
Homopolymer tailing is a method for joining two DNA molecules by adding complementary nucleotide strings. If the cloning vector is modified with a string of guanine residues using terminal transferase and dGTP, the insert DNA must be modified with a string of cytosine residues using dCTP. This allows the two molecules to hybridize through base pairing, facilitating the stable formation of recombinant DNA molecules.

**127.** (A) It is extremely labor-intensive and low-throughput
Microinjection is an incredibly efficient method for delivering DNA directly into the nucleus of a cell, but it has significant practical limitations. The process requires expensive micromanipulation equipment and a highly skilled operator to manually inject each individual cell. Because of this, the technique is very labor-intensive and has a low throughput, making it unsuitable for experiments requiring the transformation of large numbers of cells.

**128.** (A) Chloroplast genomes and recalcitrant monocots
The biolistic method, or gene gun, is a powerful tool for plant transformation that involves shooting DNA-coated microprojectiles into plant tissues. This technique is particularly valuable for species that are resistant to Agrobacterium infection, such as many important monocot crops. Additionally, it is the primary method used for transforming chloroplast genomes, as the particles can physically penetrate the multiple membranes of these organelles.

**129.** (A) The stringency of the washing buffer (salt concentration and temperature)
In colony hybridization, the specificity of the probe binding to the target DNA is controlled by the stringency of the washing conditions. Stringency is determined by the salt concentration and the temperature of the buffer used after hybridization. High stringency, achieved with low salt and high temperature, ensures that the probe only remains bound to sequences with high complementarity, reducing non-specific signals.

**130.** (C) A is true but R is false.
PCR-based site-directed mutagenesis is a common technique that uses primers containing a desired mutation to amplify a plasmid. While this process introduces the mutation, the resulting PCR products must still be transformed into a host organism, such as E. coli, to be biological amplified and expressed. Therefore, while the method uses PCR, it does not replace the need for a host for protein production.

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**131.** (A) A selectable marker flanked by sequences homologous to the target locus
Targeted gene disruption in yeast relies on the efficient process of homologous recombination. To knockout a gene, researchers design a DNA construct containing a selectable marker, such as an antibiotic resistance or auxotrophic gene. This marker is flanked by DNA sequences that are identical to the regions surrounding the target gene. Upon transformation, the yeast cell's natural machinery replaces the target gene with the marker.

**132.** (A) Knockin
A knockin is a genetic engineering technique where a functional DNA sequence is inserted into a specific, targeted location within an organism's genome. This is often used to introduce a new gene, replace a mutant gene with a healthy version, or add a reporter gene under the control of an endogenous promoter. The resulting organism typically exhibits a gain-oF-function phenotype related to the inserted sequence.

**133.** (A) It is degraded or discarded from the RISC complex
During the process of RNA interference, a double-stranded siRNA is loaded into the RNA-induced silencing complex. The complex then selects one strand, known as the guide strand, to identify target messenger RNA. The other

encoding a specific protein of interest is inserted into a bacteriophage genome, fused to the gene for one of the phage's coat proteins. As the phage replicates, it expresses the fusion protein on its surface, allowing for the direct linkage between the displayed protein's phenotype and its underlying genotype.

**82.** (A) Biopanning
Biopanning is the iterative selection process used in phage display to identify high-affinity binding molecules from a vast library of variants. A library of phages is incubated with a target antigen, and non-binding phages are washed away. The bound phages are then eluted and amplified in bacteria. Through multiple rounds of this enrichment process, phages displaying the strongest binders are systematically identified and isolated.

**83.** (B) The variable regions of heavy (VH) and light (VL) chains connected by a flexible linker
A single-chain variable fragment is a recombinant protein engineered to retain the antigen-binding specificity of a whole antibody. It consists of the variable regions of the heavy and light chains, which are fused together by a short, flexible peptide linker. This small molecule is highly stable and can be produced easily in bacterial systems, making it a versatile tool for diagnostics and targeted therapy.

**84.** (A) Reduce immunogenicity when administered to humans
Humanized antibodies are created by replacing as much of a mouse monoclonal antibody as possible with human sequences while maintaining the original antigen-binding specificity. This is typically achieved by grafting the mouse complementarity determining regions onto a human antibody framework. The primary goal is to reduce the risk of an immune response when these therapeutic agents are administered to human patients for treatment.

**85.** (A) I, II, III and V
Recombinant human insulin was the first therapeutic protein produced using genetic engineering. The standard method involves expressing the insulin A and B chains separately in E. coli as fusion proteins. After purification and cleavage of the fusion partners, the two chains are mixed and chemically oxidized to form the correct disulfide bonds. This process yields a pure protein that lacks the C-peptide.

**86.** (A) They possess absolutely zero risk of reverting to virulence
Subunit vaccines contain only specific, purified components of a pathogen, such as viral proteins or bacterial polysaccharides. Because they do not contain the entire pathogen or its genetic material, they cannot replicate or cause the disease they are designed to prevent. This eliminates the risk of reversion to a virulent form, which is a rare but serious concern with traditional live-attenuated vaccines.

**87.** (A) Random mutagenesis followed by rigorous screening
Before the development of modern genetic engineering, industrial strain improvement relied heavily on traditional methods. Researchers exposed microorganisms to physical or chemical mutagens to induce random changes in their genome. This was followed by high-throughput screening to identify rare variants with improved traits, such as increased metabolite production or faster growth rates, which were then used for large-scale industrial fermentation processes.

**88.** (A) Deleting competing metabolic pathways
Metabolic engineering is the practice of optimizing genetic and regulatory processes within cells to increase the production of specific metabolites. A common strategy involves identifying and deleting genes that lead to competing pathways, which would otherwise divert carbon sources and energy away from the desired product. This refocusing of cellular resources significantly enhances the overall yield and efficiency of the microbial production system.

**89.** (A) Proteins for animal or human consumption
Single-cell protein refers to the edible biomass of microorganisms, including bacteria, fungi, and algae, that is used as a high-protein supplement for human or animal nutrition. These organisms grow rapidly on various substrates and contain significant amounts of essential amino acids and vitamins. SCP production is considered a sustainable way to meet increasing global protein demands while utilizing industrial or agricultural waste products.

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cell, researchers can significantly improve the overall yield and productivity of industrial fermentations.

**142.** (A) Nucleic acids
Single-cell protein derived from bacteria often contains high levels of nucleic acids, particularly RNA, due to the rapid growth rates of the cells. When humans consume excessive amounts of nucleic acids, the breakdown product, uric acid, can accumulate in the body and lead to conditions like gout or kidney stones. Therefore, SCP intended for human consumption must undergo processing to reduce its nucleic acid content.

**143.** (A) Leaky vasculature and poor lymphatic drainage
Solid tumors exhibit a unique physiological environment that facilitates the accumulation of nanocarriers, a phenomenon known as the enhanced permeability and retention effect. The tumor's blood vessels are leaky and disorganized, allowing small particles to escape into the tissue. Furthermore, the lack of functional lymphatic vessels prevents these particles from being cleared, resulting in higher local concentrations of the therapeutic agent compared to healthy tissues.

**144.** (B) II
Genetically modified crops are engineered for diverse traits. Bt cotton provides insect resistance, and herbicide-tolerant crops often carry a modified EPSPS gene to survive glyphosate application. In India, the GEAC regulates these crops. However, Golden Rice is specifically designed to produce high levels of pro-vitamin A, or beta-carotene, to address deficiencies, rather than Vitamin $B_{12}$ as suggested in the incorrect statement.

**145.** (A) Produce a measurable signal indicating spatial or temporal gene expression
A reporter gene is a tool used to study the activity of a specific promoter or the localization of a protein. When fused to a regulatory sequence, the reporter gene produces an easily detectable signal, such as fluorescence or a color change, when the gene is expressed. This allows researchers to track exactly where and when a gene is active within an organism or cell culture.

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**146.** (A) Genome walking (or Inverse PCR)
While techniques like Southern blotting can confirm the presence of a transgene, genome walking is the definitive method for identifying its exact integration site. This approach involves amplifying the unknown genomic DNA sequence adjacent to the known transgene sequence. By sequencing these flanking regions, researchers can map the precise location of the insertion within the host genome and assess potential impacts on nearby endogenous genes.

**147.** (A) The transfer of transgenes to sexually compatible wild relatives
Environmental risk assessments for genetically modified organisms focus heavily on the phenomenon of gene flow. This occurs when pollen from a transgenic crop fertilizes a sexually compatible wild relative, potentially transferring the modified trait into the natural population. Evaluating the likelihood and consequences of such transfers is essential for ensuring that the release of GMOs does not lead to unintended ecological disruptions or invasive species.

**148.** (A) Positive pressure personnel suits with segregated air supply
Biosafety Level 4 represents the highest level of biological containment. It is reserved for work with dangerous and exotic agents that pose a high risk of life-threatening disease for which there are no treatments or vaccines. Personnel must work in completely sealed laboratories and wear positive pressure suits with a dedicated, segregated air supply to ensure absolute protection from accidental exposure to pathogens.

**149.** (A) The safe handling, transport, and use of living modified organisms (LMOs)
The Cartagena Protocol on Biosafety is an international treaty that governs the transboundary movement of living modified organisms resulting from modern biotechnology. Its primary goal is to protect biological diversity from potential risks posed by LMOs by establishing rules for their safe handling, transport, and use. The protocol emphasizes the precautionary principle, allowing countries to make informed decisions about importing these organisms into their territories.

protect the surrounding environment from accidental release of the pathogens.

**99.** (C) Genetic Engineering Appraisal Committee (GEAC)
In India, the Genetic Engineering Appraisal Committee is the apex regulatory body responsible for the environmental release of genetically modified organisms. Operating under the Ministry of Environment, Forest and Climate Change, it reviews and approves large-scale field trials and the commercialization of transgenic crops. The committee ensures that all biotech products meet stringent safety and environmental standards before they reach the public market.

**100.** (A) P-1, Q-3, R-2, S-4
The regulatory framework for biotechnology in India involves multiple committees. The Institutional Biosafety Committee monitors local experiments, whereas the Review Committee on Genetic Manipulation evaluates small-scale confined trials. The Genetic Engineering Appraisal Committee holds the authority to approve large-scale trials and commercial releases, and District Level Committees ensure compliance with these regulations at the local level.

**101.** (B) Potential for transgene flow to wild relatives
A primary concern in the environmental risk assessment of genetically modified organisms is the possibility of gene flow. This refers to the transfer of the inserted transgene from the GM crop to sexually compatible wild relatives through cross-pollination. Such events could potentially lead to the creation of invasive weeds or disrupt local ecosystems, making it a critical factor in regulatory safety evaluations.

**102.** (A) Non-obviousness (inventive step)
To be granted a patent, an invention must meet three fundamental legal criteria. First, it must be novel, meaning it has not been disclosed previously. Second, it must have industrial applicability. Finally, it must involve an inventive step, which means the modification or discovery must not be obvious to a person with ordinary skill in the relevant field of technology at the time.

**103.** (A) The deposition of microorganisms in recognized culture collections
The Budapest Treaty is an international agreement that simplifies the patent process for inventions involving microorganisms. It allows an inventor to deposit a sample of the microorganism in a single recognized international depository authority. This deposit satisfies the requirement for a full written description of the organism in all member countries, ensuring that the biological material is preserved and accessible for patent verification.

**104.** (B) New, involve an inventive step, and are capable of industrial application
The Agreement on Trade-Related Aspects of Intellectual Property Rights mandates that all member countries provide patent protection for both products and processes. Patents must be available for inventions in all fields of technology, provided they are new, involve a significant inventive step, and have a clear industrial application. This international standard ensures a consistent level of intellectual property protection across global markets.

**105.** (B) Ensuring sequence accuracy of the inserted gene
Ethical discussions regarding genetically modified organisms focus on broader societal and environmental impacts. Common concerns include the potential for interfering with natural processes, the risk of allergenicity in food, and the economic influence of large corporations on the global seed supply. While maintaining sequence accuracy is a technical and regulatory requirement, it is generally considered a matter of quality control rather than an ethics issue.

**106.** (A) 2.0
The purity of nucleic acid preparations is frequently assessed using ultraviolet spectrophotometry. For highly pure RNA, the ratio of absorbance at 260 nm to that at 280 nm is approximately 2.0. A ratio significantly lower than this value often indicates the presence of protein or phenol contamination, while a higher ratio might suggest the presence of residual reagents from the isolation process.

**107.** (A) It involves a highly cooperative transition resulting in a sigmoidal melting curve
The denaturation of double-stranded DNA is a highly cooperative process. As heat is

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fragments, which is a fundamental requirement for most recombinant DNA applications.

**159.** (A) Recognize the same sequence but cleave at different positions
Neoschizomers are a specific class of isoschizomers. While all isoschizomers recognize the same DNA sequence, neoschizomers are distinguished by the fact that they cleave the DNA at different positions within that site. For example, one enzyme might produce a blunt end while the other produces a sticky end. This variability gives researchers flexibility in choosing the most appropriate enzyme for their specific cloning strategy.

**160.** (A) IV, II, I, III
PCR-based site-directed mutagenesis follows a logical progression. First, mutagenic primers containing the desired base change are designed. These primers are then used in a PCR reaction to amplify the entire plasmid, incorporating the mutation into the new strands. The product is treated with DpnI to degrade the methylated parental DNA. Finally, the mutated plasmid is transformed into E. coli to be propagated and verified.

**161.** (A) Long, full-length cDNAs
In the synthesis of cDNA, the RNase H activity of reverse transcriptase can sometimes lead to the premature degradation of the RNA template, especially in regions with complex secondary structures. This can result in truncated cDNA molecules. Using a reverse transcriptase that has been engineered to lack RNase H activity helps to maintain the integrity of the RNA template, facilitating the synthesis of long, full-length cDNA transcripts.

**162.** (A) Origin of replication (e.g., rop gene mutation)
The copy number of a plasmid is primarily controlled by the regulatory mechanisms at its origin of replication. In many vectors, such as pBR322, the Rop protein acts as a negative regulator of replication. By mutating or deleting the rop gene, the inhibitory control is removed, allowing the plasmid to replicate to a much higher copy number per cell. This increases the total yield of cloned DNA.

**163.** (A) 20 kb
Bacteriophage lambda replacement vectors are designed to carry large fragments of foreign DNA. They are constructed by removing a non-essential central portion of the viral genome, known as the stuffer fragment. This allows the vector to accommodate DNA inserts up to approximately 20 to 25 kilobases. The resulting recombinant molecules are then packaged into infectious phage particles for highly efficient delivery into the host bacteria.

**164.** (A) It can be manipulated as a plasmid and packaged as single-stranded DNA upon helper phage infection
Phagemids are versatile hybrid vectors that combine the ease of plasmid manipulation with the utility of filamentous phage. Under normal conditions, they replicate as double-stranded plasmids. However, if the host cell is co-infected with a helper phage, the phagemid's viral origin is activated. This results in the production of single-stranded DNA, which is then packaged into phage particles and secreted, providing a pure template for sequencing.

**165.** (A) Conjugation and transfer of genetic material
The $F^-$plasmid, or fertility factor, is a naturally occurring large plasmid in E. coli that plays a central role in horizontal gene transfer through conjugation. It carries genes that allow the bacterium to form a pilus and transfer DNA to a recipient cell. The replication and partition machinery of the $F^-$plasmid is highly stable, which provided the biological foundation for developing bacterial artificial chromosomes.

**166.** (A) Integrate specifically at chromosome 19q13.4 in the human genome
Adeno-associated virus is a unique viral vector that is widely studied for gene therapy due to its low immunogenicity and ability to provide long-term expression. In its wild-type form, AAV has the remarkable ability to integrate its DNA site-specifically into a particular locus on human chromosome 19. While recombinant AAV vectors typically remain episomal, this natural targeting ability highlights the virus's sophisticated relationship with its host genome.

**167.** (A) $GAL_1$ promoter
The $GAL_1$ promoter is one of the most commonly used inducible systems in Saccharomyces cerevisiae. It is highly active in the presence of galactose but is tightly repressed when the cells are grown on glucose. This allows researchers to grow yeast cultures under non-inducing conditions

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**184.** (A) Polyethylene glycol (PEG)
Protoplast fusion is a method used to combine the genetic material of two different plant cells that are otherwise sexually incompatible. After the cell walls are enzymatically removed, the resulting protoplasts are treated with polyethylene glycol. This chemical acts as a fusogen, inducing the membranes of adjacent protoplasts to merge. This process creates hybrid cells that can be regenerated into entire plants, carrying traits from both parent species.

**185.** (A) RNA-dependent RNA polymerase
Reverse transcriptase is a multi-functional enzyme derived from retroviruses. Its primary activities include RNA-dependent DNA polymerase, which synthesizes the first cDNA strand, and DNA-dependent DNA polymerase, which helps form the second strand. Additionally, it possesses RNase H activity to degrade the RNA template within an RNA-DNA hybrid. Crucially, it does not possess RNA-dependent RNA polymerase activity, which is typically found in certain RNA viruses for genome replication.

**186.** (A) 5'-GAATTC-3'
Type II restriction enzymes typically recognize palindromic DNA sequences, which read the same in the 5' to 3' direction on both strands. The sequence 5'-GAATTC-3' is a classic example, recognized by the enzyme EcoRI. Its complementary strand also reads 5'-GAATTC-3' when read in the opposite direction. This symmetry allows the enzyme to bind and cut both strands at specific, predictable locations, making it a fundamental tool in recombinant DNA technology.

**187.** (A) Logarithm of its molecular weight
During agarose gel electrophoresis, the migration of linear double-stranded DNA fragments is influenced by their size. Because the gel matrix acts as a molecular sieve, smaller fragments move faster through the pores than larger ones. Specifically, the migration rate is inversely proportional to the logarithm of the molecular weight of the DNA. This mathematical relationship allows researchers to estimate the size of unknown DNA fragments by comparing them to a known marker.

**188.** (A) A mixture of Taq polymerase and a proofreading polymerase
Long-range PCR is a specialized technique designed to amplify DNA fragments that are much larger than the typical five to ten kilobase limit of standard PCR. This is achieved by using a balanced mixture of Taq DNA polymerase and a second polymerase that possesses 3' to 5' proofreading exonuclease activity. The proofreading enzyme repairs base-incorporation errors that would otherwise cause Taq to stall, allowing for the successful synthesis of very long DNA strands.

**189.** (A) $ColE_1$ origin and ampicillin resistance gene
Shuttle vectors are engineered to replicate in two different host organisms, such as yeast and E. coli. For a yeast artificial chromosome to function as a shuttle vector, it must contain elements necessary for both hosts. While it requires yeast-specific sequences like telomeres and centromeres, it must also include a bacterial origin of replication, such as $ColE_1$, and a selectable marker like the ampicillin resistance gene to allow for propagation and selection in E. coli.

**190.** (A) Linear double-stranded DNA
Adenoviruses are common human pathogens that have been widely adapted as viral vectors for gene delivery. The adenovirus genome consists of a large, linear molecule of double-stranded DNA, typically around thirty-six kilobases in length. In recombinant versions, the viral genes responsible for replication are often removed to provide space for large therapeutic genes and to ensure that the virus cannot replicate once it enters the target host cell.

**191.** (A) Cause insertional mutagenesis by integrating near proto-oncogenes
Retroviral vectors integrate their genetic material randomly into the host genome to achieve stable expression. However, this process carries a significant safety risk known as insertional mutagenesis. If the vector integrates near a cellular proto-oncogene, it can inadvertently activate the gene, potentially leading to uncontrolled cell growth and the development of cancer. This concern has led to the development of safer, selF$^-$inactivating vectors for human gene therapy trials.

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**176.** (A) They are uncharged and highly resistant to nucleolytic degradation
Morpholinos are synthetic molecules widely used for gene silencing because of their exceptional stability. Their unique structure replaces the ribose sugar with a morpholine ring and utilizes non-ionic phosphorodiamidate linkages. Because they are uncharged, they do not interact non-specifically with cellular proteins. Furthermore, they are completely resistant to all known nucleases, ensuring that they remain functional within the cell for much longer than traditional DNA or RNA-based oligonucleotides.

**177.** (A) Entire human immunoglobulin gene loci into a mouse whose endogenous loci are inactivated
To produce completely human monoclonal antibodies, researchers have engineered transgenic mice. This involves inactivating the mouse's own immunoglobulin genes and replacing them with large segments of the human antibody loci. When these mice are immunized, their B-cells produce fully human antibodies rather than mouse or chimeric versions. This technology eliminates the need for subsequent humanization steps and significantly reduces the immunogenicity of the resulting therapeutic products.

**178.** (A) Risk of transmitting Creutzfeldt-Jakob disease (prions)
Before recombinant technology, human growth hormone was extracted from the pituitary glands of human cadavers. However, this practice was halted after it was discovered that some recipients developed Creutzfeldt-Jakob disease, a fatal neurodegenerative condition caused by prions. The advent of recombinant human growth hormone produced in E. coli eliminated this risk entirely, providing a safe and consistent supply of the hormone for children with growth deficiencies.

**179.** (A) Biocompatible and biodegrade into naturally occurring metabolic products
Polymeric nanocarriers like PLGA are highly valued in drug delivery because of their excellent safety profile. PLGA is both biocompatible, meaning it does not elicit a significant immune response, and biodegradable. Inside the body, it undergoes hydrolysis to form lactic acid and glycolic acid, which are naturally occurring metabolic products that are easily cleared. This allows for the controlled release of encapsulated drugs without leaving harmful synthetic residues in the tissue.

**180.** (A) P-4, Q-1, R-2, S-3
Genetically modified organisms have been developed for various agricultural and nutritional purposes. The Flavr Savr tomato was designed for delayed ripening using antisense technology. Roundup Ready soybeans carry a gene that provides resistance to glyphosate herbicides. Bt cotton expresses Cry proteins to provide insect resistance, and Golden Rice is engineered to accumulate beta-carotene, a precursor to vitamin A, addressing nutritional deficiencies in global populations.

**181.** (A) 20 years
The TRIPS Agreement is an international legal treaty that sets minimum standards for intellectual property regulation. One of its key provisions is that all member nations must provide a patent term of at least twenty years from the date the application is filed. This duration is intended to provide inventors with a sufficient period of exclusivity to recoup their research and development costs while encouraging the disclosure of new technologies to the public.

**182.** (A) Vigorous vortexing or physical shearing
Isolating high-molecular-weight genomic DNA requires careful handling to maintain the integrity of the long DNA molecules. Physical stress, such as vigorous vortexing or rapid pipetting, creates shear forces that can easily break the large DNA strands into smaller fragments. To obtain pure, intact genomic DNA suitable for large-fragment cloning or long-read sequencing, researchers must use gentle mixing techniques and wide-bore pipette tips throughout the entire extraction process.

**183.** (A) PstI
Restriction endonucleases produce different types of DNA ends based on their cleavage pattern. While some enzymes produce 5' overhangs or blunt ends, PstI is a well-known example that generates 3' protruding cohesive ends. It recognizes the palindromic sequence 5'-CTGCAG-3' and cuts between the adenine and guanine on each strand. These 3' sticky ends are useful in molecular cloning for directional insertion and for certain tailing reactions.

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modification, making it ineligible for patent protection. In contrast, a novel genetically engineered bacterium, a new chemical process for DNA synthesis, or a specific synthetic vector sequence involve human intervention and ingenuity, meeting the criteria for novelty and non-obviousness required for a patent to be granted.

**233.** (A) 50 μg/mL
Spectrophotometry is the standard method for quantifying DNA concentration. For double-stranded DNA, an absorbance of 1.0 at 260 nanometers corresponds to a concentration of approximately fifty micrograms per milliliter. This relationship is based on the known extinction coefficient of DNA. For single-stranded DNA or RNA, the values are different, with an OD260 of 1.0 corresponding to approximately thirty-three and forty micrograms per milliliter, respectively, allowing for quick and accurate quantification.

**234.** (A) Disrupting hydrogen bonds between base pairs
Formamide is a chemical denaturant frequently added to hybridization buffers to lower the melting temperature of double-stranded nucleic acids. It works by competing for and disrupting the hydrogen bonds that hold the complementary base pairs together. By adding formamide, researchers can conduct hybridization and stringency washes at lower temperatures, which helps to preserve the integrity of the RNA or DNA molecules and the membrane while maintaining high sequence specificity during the experiment.

**235.** (A) The sizes of DNA fragments produced after single and double digests
Restriction mapping is a technique used to determine the relative locations of restriction enzyme recognition sites on a DNA molecule. It involves digesting the DNA with several different enzymes, both individually and in combination. By analyzing the resulting fragment sizes resolved by gel electrophoresis, researchers can logically deduce the arrangement and distance between the cut sites. This map provides a structural blueprint of the DNA, which is essential for cloning and sequence verification.

**236.** (A) 5' to 3' only
All known DNA polymerases catalyze the synthesis of new DNA strands exclusively in the 5' to 3' direction. The enzyme works by adding a deoxynucleotide to the free 3'-hydroxyl group of the growing strand, forming a phosphodiester bond. This biochemical requirement means that the new strand is always synthesized starting from its 5' end toward its 3' end. Consequently, the polymerase must read the template strand in the 3' to 5' direction.

**237.** (A) Allows monitoring of amplification during the exponential phase using fluorescent probes
Quantitative PCR differs from standard PCR because it allows for the real-time monitoring of DNA amplification. The reaction includes fluorescent dyes or probes that emit a signal proportional to the amount of DNA produced. By detecting this signal during the exponential phase of the reaction, researchers can accurately quantify the initial amount of target DNA in the sample. This high sensitivity and precision make qPCR an essential tool for measuring gene expression and detecting pathogens.

**238.** (A) Using two different incompatible restriction enzymes to ensure the insert ligates in only one specific orientation
Directional cloning is a strategy used to ensure that a DNA fragment is inserted into a vector in a specific, predetermined orientation. This is achieved by digesting both the insert and the vector with two different restriction enzymes that produce incompatible sticky ends. Because the ends can only hybridize in one way, the insert is forced to ligate in the correct orientation. This is particularly important for expression cloning, where the gene must be correctly aligned with the promoter.

**239.** (A) $T_7$ RNA polymerase
The pET expression system is designed for the high-level production of recombinant proteins in E. coli. It utilizes the $T_7$ promoter, which is specifically recognized by the $T_7$ RNA polymerase rather than the host's endogenous RNA polymerase. To express the target gene, the host bacteria must be engineered to carry the gene for $T_7$ RNA polymerase, usually under the control of an inducible promoter like lac, allowing for controlled and massive protein synthesis.

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**192.** (A) Pichia pastoris
Pichia pastoris has emerged as a premier host for the industrial production of recombinant proteins. It combines the ease of bacterial culture with the complex post-translational modifications typical of eukaryotic cells. Furthermore, Pichia can be grown to extremely high cell densities in large-scale fermenters and possesses a highly efficient secretory pathway. These characteristics, along with the strong AOX1 promoter, allow for the production of massive quantities of pure, functional proteins.

**193.** (A) Glutathione sepharose
The glutathione S-transferase tag is a large protein tag used to enhance the solubility and facilitate the purification of recombinant proteins. The GST tag has a strong and specific affinity for glutathione. During purification, the tagged protein is passed through a column containing glutathione sepharose beads. After washing away contaminants, the purified protein is eluted by adding free glutathione, which competes for the binding sites on the resin.

**194.** (A) Empty phage heads and tail assemblies
In vitro viral packaging is a method for introducing recombinant lambda or cosmid DNA into bacteria with high efficiency. The process requires a concentrated extract of phage proteins, including empty phage heads and pre-assembled tail structures. When the recombinant DNA is mixed with these extracts, the DNA is automatically packaged into the heads, and the tails are attached, forming functional, infectious phage particles that can deliver the DNA into host cells.

**195.** (A) $CaCl_2$
Preparing competent E. coli cells involves treating the bacteria with a cold solution of calcium chloride. The divalent calcium ions are thought to neutralize the negative charges on the DNA and the cell membrane, allowing the DNA to associate closely with the cell surface. This treatment, combined with a brief incubation on ice, permeabilizes the membrane, preparing the cells for the subsequent heat shock that drives the DNA into the cytoplasm.

**196.** (A) I, II and III
Blue-white screening is an effective way to identify recombinant bacterial colonies. The process requires a host cell with a specific deletion in the lacZ gene and a vector carrying the alpha peptide. When IPTG is added as an inducer, the host and vector fragments complement each other to form active beta-galactosidase, which cleaves X-gal into a blue product. Recombinant colonies, where the vector's alpha peptide gene is disrupted, appear white.

**197.** (A) Random point mutations via guanine alkylation
Ethyl methanesulfonate is a powerful chemical mutagen widely used in genetic research. It functions by alkylating guanine residues, typically resulting in the formation of O6-ethylguanine. During DNA replication, this modified base pairs with thymine instead of cytosine, leading to permanent G/C to A/T transition mutations. This process generates a vast library of random point mutations across the genome, allowing researchers to study the effects of individual amino acid changes.

**198.** (A) Post-transcriptional gene silencing
RNA interference is a biological process in which small RNA molecules inhibit gene expression. Because it functions by targeting and degrading specific messenger RNA transcripts after they have been transcribed from the DNA, it is classified as a form of post-transcriptional gene silencing. This natural mechanism allows the cell to regulate gene activity rapidly and precisely, and it has been adapted into a powerful tool for experimental gene knockdown.

**199.** (A) 21-23 bp double-stranded RNA with 2-nucleotide 3' overhangs
Synthetic small interfering RNAs are designed to mimic the natural products of Dicer cleavage. They typically consist of a short, double-stranded RNA molecule twenty-one to twenty-three base pairs in length. A characteristic feature is the presence of two-nucleotide long overhangs at the 3' end of each strand. This specific structure is essential for the siRNA to be correctly recognized and loaded into the RNA-induced silencing complex for targeted mRNA degradation.

**200.** (A) Smaller size allowing better tissue penetration
Single-chain variable fragments offer several advantages over whole monoclonal antibodies in medical applications. Because they consist only of the essential antigen-binding regions connected by a linker, they are significantly smaller than intact IgG molecules. This reduced

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and then switch to galactose-containing media to trigger the high-level expression of a recombinant gene at a specific developmental stage.

**168.** (A) P-3, Q-1, R-4, S-2
A functional expression vector requires several integrated components. The origin of replication ensures that the vector replicates within the host. The promoter drives the transcription of the inserted gene, while a selectable marker allows for the identification of cells containing the plasmid. Finally, a His-tag can be fused to the protein to facilitate easy one-step purification using immobilized metal affinity chromatography after the protein is expressed.

**169.** (A) Short, blunt-ended, double-stranded synthetic DNA containing a restriction site
Linkers are synthetic, double-stranded oligonucleotides designed to facilitate cloning. They are typically blunt-ended and contain a specific internal recognition sequence for a restriction enzyme. When ligated to the ends of a blunt DNA fragment, they can be subsequently digested to create cohesive sticky ends. This allows the fragment to be easily inserted into a vector that has been prepared with complementary overhangs, improving overall cloning efficiency.

**170.** (A) Both A and R are true and R is the correct explanation of A.
In vitro viral packaging significantly improves the efficiency of introducing recombinant lambda DNA into bacteria compared to traditional chemical transformation. The process mimics natural infection, where the phage particle protects the DNA from environmental degradation and then actively injects it into the host cell. This biological delivery mechanism is much more effective than forcing DNA through chemically induced pores in the cell membrane during transformation.

**171.** (A) Endocytosis of lipid-DNA complexes
Lipofection is a popular method for delivering genetic material into eukaryotic cells. It utilizes cationic lipids that form small, artificial vesicles called liposomes, which encapsulate the negatively charged DNA. These lipid-DNA complexes have a high affinity for the cellular membrane. Once they bind, the complexes are internalized by the cell through the process of endocytosis, eventually releasing the DNA into the cytoplasm or nucleus for expression.

**172.** (A) White on X-gal plates
The blue-white screening method utilizes the disruption of the lacZ alpha peptide gene to identify recombinant clones. If the vector is non-recombinant, the functional beta-galactosidase enzyme is produced, which cleaves X-gal to form blue colonies. However, when an insert is successfully ligated into the multiple cloning site, the gene is inactivated. This prevent the formation of a functional enzyme, resulting in colonies that appear white on X-gal plates.

**173.** (A) Primers containing the desired mutation flanked by complementary sequences
Primer extension mutagenesis is a technique used to introduce specific changes into a DNA sequence. A synthetic oligonucleotide primer is designed with the desired mutation, surrounded by sequences that are perfectly complementary to the template. During DNA synthesis, the primer hybridizes to the template, and the polymerase extends it, incorporating the mutation into the newly synthesized strand. This method allows for precise, site-specific changes within a cloned gene.

**174.** (A) Targeted gene disruption (Knockout)
Targeted gene disruption, commonly known as a knockout, is a fundamental technique in functional genomics. It involves using a DNA construct that contains a non-functional version of a gene flanked by sequences homologous to the target locus. Through the process of homologous recombination, the organism's natural DNA repair machinery replaces the wild-type gene with the defective copy, effectively eliminating the expression and function of that specific gene.

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**175.** (A) Assist Dicer and recruit the $AgO_2$ protein to form the RISC
The TAR RNA-binding protein is a critical cofactor in the human RNA interference pathway. It forms a complex with the Dicer enzyme to facilitate the processing of long double-stranded RNA into small interfering RNAs. Beyond processing, TRBP plays a vital role in recruiting the Argonaute 2 protein to the mature siRNA, thereby assisting in the assembly of the functional RNA-induced silencing complex, which executes target mRNA degradation.

RNase A may contain trace amounts of DNase, which would degrade the target plasmid DNA. Because RNase A is extremely heat-stable and can renature after boiling, while most DNases are permanently inactivated by heat, boiling the enzyme solution is a standard procedure to ensure it is safe to use in DNA isolation protocols.

**209.** (A) 5' to 3' exonuclease
The Klenow fragment is produced by the enzymatic digestion of E. coli DNA polymerase I with subtilisin. This process removes the small N-terminal domain, which contains the 5' to 3' exonuclease activity. The resulting large fragment retains both the 5' to 3' polymerase and the 3' to 5' proofreading exonuclease activities. This makes it a valuable tool for applications like filling in recessed 3' ends or DNA sequencing where 5' degradation is undesirable.

**210.** (A) Compatible sticky ends
Isoschizomers are restriction enzymes that recognize the same DNA sequence. When two isoschizomers cleave at the exact same position within that sequence, they produce identical terminal structures. These fragments possess compatible sticky ends, allowing them to be easily ligated together. This property provides researchers with flexibility, enabling them to use different enzymes to achieve the same cloning result depending on availability or specific reaction conditions like temperature and buffer requirements.

**211.** (A) SYBR Green
SYBR Green is a highly sensitive fluorescent dye that has become a popular, safer alternative to ethidium bromide for staining nucleic acids in gels. It binds specifically to the minor groove of double-stranded DNA. Unlike ethidium bromide, which is a potent mutagen, SYBR Green is significantly less hazardous to work with. Furthermore, it exhibits much lower background fluorescence and provides a higher signal-to-noise ratio, improving the detection of low-abundance DNA fragments.

**212.** (A) Gene-specific primer
When the goal is to synthesize a full-length cDNA for a specific, known mRNA, a gene-specific primer is the most appropriate choice. Unlike random hexamers or oligo(dT) primers, which target all mRNA molecules, a gene-specific primer hybridizes only to the sequence of interest. This high specificity ensures that the reverse transcriptase initiates synthesis exclusively from the target transcript, maximizing the yield of the desired cDNA and reducing the complexity of the resulting mixture.

**213.** (A) Is packaged into phage heads in vitro via its cos sites
Cosmids are hybrid vectors that combine the features of plasmids and bacteriophage lambda. They contain a bacterial origin of replication and a selectable marker, but they also include the lambda cos sites. These sites allow the cosmid to be packaged in vitro into phage heads, provided the insert DNA is of the appropriate size. Once inside the host cell, the cosmid circularizes and replicates as a large, stable plasmid.

**214.** (A) BAC
Bacterial artificial chromosomes are designed to maintain large genomic inserts with high stability. They utilize the replication system of the E. coli $F^-$ plasmid, which strictly regulates the vector to a single copy per cell. This low copy number is crucial for preventing homologous recombination and other structural rearrangements that often occur with large inserts in high-copy plasmids. This stability makes BACs the preferred choice for constructing large-insert libraries and physical mapping.

**215.** (A) It provides proteins necessary for AAV replication
Adeno-associated virus is a defective virus that cannot replicate on its own. In a recombinant AAV production system, a helper virus, such as adenovirus or herpes simplex virus, is required to provide essential genes. These helper functions include proteins that facilitate AAV DNA replication and the assembly of the viral capsids. Alternatively, these helper genes can be provided by a separate plasmid in a triple-transfection method, allowing for the production of safe, replication-deficient vectors.

**216.** (A) P-1, Q-4, R-2, S-3
Mutagenesis techniques offer various ways to alter DNA. Chemical mutagenesis uses agents like EMS for global point mutations, while cassette mutagenesis replaces specific restriction fragments with synthetic mutant sequences. PCR-based methods utilize mismatched primers to introduce targeted changes. DNA shuffling is a unique approach that fragments homologous

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size allows them to penetrate dense tissues, such as solid tumors, more effectively. Additionally, they are easier to produce in bacterial expression systems and have a faster clearance rate from the bloodstream.

**201.** (A) Culturing hybridoma cells in stirred-tank bioreactors
The large-scale production of therapeutic monoclonal antibodies is typically achieved using stirred-tank bioreactors. Hybridoma cells or engineered mammalian cell lines, such as Chinese Hamster Ori cells, are grown in large volumes under carefully controlled conditions. This scalable approach allows for the consistent production of grams to kilograms of high-purity antibodies. The process includes sophisticated aeration and nutrient supply systems to maintain high cell viability and maximize overall protein yield.

**202.** (A) Microalgae, fungi, or bacteria
Single-cell protein is a sustainable food source derived from the biomass of microorganisms like microalgae, fungi, or bacteria. These organisms are grown on various substrates, including industrial side-streams, and harvested for their high protein content. SCP is rich in essential amino acids and can be produced independently of climate conditions. It serves as an alternative to traditional animal-based proteins, helping to address global food security and reduce the environmental footprint of protein production.

**203.** (A) Polyethylene glycol (PEG)
Stealth liposomes are specialized drug delivery vesicles that have been surface-modified with polyethylene glycol. This PEG coating creates a steric barrier that prevents the liposomes from being recognized and cleared by the reticuloendothelial system, particularly the macrophages in the liver and spleen. By avoiding immune detection, stealth liposomes remain in the bloodstream for a significantly longer period, allowing them to accumulate more effectively at target sites like tumors.

**204.** (A) Both A and R are true and R is the correct explanation of A.
Agrobacterium tumefaciens is a highly efficient natural genetic engineer for dicotyledonous plants. When a plant is wounded, it releases specific phenolic signaling molecules, such as acetosyringone. These compounds are sensed by the bacterium, which then activates its virulence genes. This triggers the transfer of a specific T-DNA segment into the plant cell, providing a reliable mechanism that scientists have adapted for the genetic transformation of diverse dicot species.

**205.** (A) Ministry of Environment, Forest and Climate Change
In India, the Genetic Engineering Appraisal Committee is the statutory body responsible for regulating the environmental release of genetically modified organisms. It functions under the Ministry of Environment, Forest and Climate Change. The committee's mandate includes evaluating the potential impacts of GMOs on human health and the environment before granting approval for large-scale field trials or commercial cultivation, ensuring that biotechnology is used safely and responsibly.

**206.** (A) Not patentable
The Indian Patent Act contains specific provisions regarding the patentability of biological materials. According to Section 3(j), plants and animals in their entirety, or any part thereof, including seeds, varieties, and species, are not considered patentable inventions. While microorganisms and specific biotechnological processes may be eligible for patent protection, the law explicitly excludes higher organisms to protect traditional agricultural practices and ensure that life forms are not treated as mere commodities.

**207.** (A) It eliminates secondary structures like hairpins
Denaturation of RNA involves the disruption of the hydrogen bonds that maintain its complex secondary and tertiary structures, such as hairpins and loops. This process is typically achieved using heat or chemical denaturants like formamide. Unlike DNA denaturation, which involves strand separation, RNA denaturation simply unfolds the single-stranded molecule into a linear configuration. This is essential for accurate size determination during gel electrophoresis and for efficient hybridization in various molecular assays.

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**208.** (A) Boiled
RNase A is a robust enzyme used during plasmid purification to remove contaminating RNA. However, commercial preparations of

Additionally, the resulting RNA-RNA duplex can be recognized and degraded by endogenous nucleases, further reducing the expression of the target gene.

**225.** (A) ~5-10% (only the CDRs)
A humanized antibody is engineered to be as similar to a human antibody as possible to minimize immunogenicity. In the humanization process, only the complementarity determining regions, which comprise about five to ten percent of the total protein sequence, are retained from the original mouse monoclonal antibody. The rest of the molecule, including the framework and constant regions, is replaced with human sequences, ensuring that the therapeutic antibody is well-tolerated by the human immune system.

**226.** (A) Hemophilia A
Recombinant factor VIII is a life-saving therapeutic protein produced using mammalian cell culture technology. It is used to treat patients with hemophilia A, a genetic disorder characterized by the lack of this essential blood-clotting factor. Before the advent of recombinant DNA, factor VIII was purified from donated human plasma, which carried a high risk of transmitting viral infections. The recombinant version provides a safe, consistent, and virus-free supply of the clotting factor for patients.

**227.** (A) Reversion to wild-type characteristics
Industrial strain improvement focuses on enhancing the performance of microorganisms used in fermentation. The goals include increasing the yield of target metabolites, improving tolerance to high product concentrations, and developing resistance to phage infections. Importantly, the process does not aim for reversion to wild-type characteristics. In fact, industrial strains are often highly specialized and genetically distinct from their natural counterparts to ensure they remain optimized for the harsh conditions of large-scale industrial production.

**228.** (A) An aqueous core surrounded by a lipid bilayer or a solid lipid core
Lipid nanoparticles are sophisticated drug delivery systems composed of various lipids, including phospholipids and cholesterol. Structurally, they often consist of an aqueous internal core surrounded by one or more lipid bilayers, similar to a liposome, or they may have a solid lipid core. This structure allows them to encapsulate both hydrophilic and hydrophobic drugs or nucleic acids, protecting them from degradation and facilitating their delivery to specific cells or tissues within the body.

**229.** (A) Kanamycin
The nptII gene, which encodes the enzyme neomycin phosphotransferase II, is one of the most widely used selectable markers in plant genetic engineering. It provides resistance to several aminoglycoside antibiotics, most notably kanamycin. When plant cells are transformed with a construct containing nptII, they can be grown on media containing kanamycin. Only the cells that have successfully integrated the transgene will survive and proliferate, allowing for the efficient selection of transgenic plant tissues and regenerated plants.

**230.** (A) Lepidopteran insect pests
Bt crops are transgenic plants that have been engineered to express Cry proteins from the soil bacterium Bacillus thuringiensis. These proteins are highly specific toxins that target the digestive systems of certain insect pests, particularly those in the order Lepidoptera, such as the bollworm. When the larvae ingest the transgenic plant tissue, the toxin is activated, leading to their death. this targeted approach reduces the need for broad-spectrum chemical insecticides, providing an effective pest management strategy.

**231.** (A) Prevent the escape and establishment of genetically modified agents in the environment
Containment protocols are a fundamental aspect of biosafety in recombinant DNA research. Their primary purpose is to provide physical and biological barriers that prevent the accidental escape of genetically modified organisms or hazardous biological agents into the outside environment. By ensuring that these agents remain confined within the laboratory or specialized greenhouse, containment minimizes the risk of unintended environmental impacts, such as the disruption of local ecosystems or the transfer of transgenes to wild species.

**232.** (A) A newly discovered naturally occurring rainforest plant
Patent law distinguishes between inventions and mere discoveries. A naturally occurring plant found in a rainforest is a product of nature and does not involve an inventive step or human

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genes and then recombines them, allowing for the rapid evolution of proteins with improved functions through the accumulation of beneficial mutations.

**217.** (A) Selectable marker complementing a host auxotrophy
In yeast genetics, the $URA_3$ gene is frequently used as a selectable marker in shuttle vectors. It encodes an enzyme required for uracil biosynthesis. When transformed into a host strain that is naturally deficient in $URA_3$, only the cells that successfully take up the plasmid will be able to grow on media lacking uracil. This auxotrophic complementation provides a robust selection mechanism that is highly effective for identifying and maintaining recombinant yeast strains.

**218.** (A) Decouple the growth phase of the culture from the protein production phase
Inducible promoters give researchers precise control over the timing of gene expression. This is particularly useful in industrial biotechnology, where high-level expression of a recombinant protein can inhibit the growth of the host cell. By using an inducer, the culture can be grown to a high density under non-expressing conditions. Once the target biomass is achieved, the promoter is activated, ensuring that cellular energy is redirected toward protein synthesis at the optimal time.

**219.** (A) I, II and IV
Northern blotting is a fundamental technique for analyzing gene expression at the RNA level. It involves separating RNA molecules by size through gel electrophoresis, transferring them to a solid membrane, and then detecting specific transcripts using a labeled nucleic acid probe. This method provides critical information about the size of the transcript and its relative abundance in different samples. Unlike Western blotting, it does not utilize antibodies for the detection of the target molecule.

**220.** (A) Forming a DNA-calcium complex precipitate that adheres to the cell and is endocytosed
Calcium phosphate transfection is a classic chemical method for delivering DNA into eukaryotic cells. When DNA is mixed with calcium chloride and a phosphate buffer, it forms a fine insoluble precipitate of calcium phosphate-DNA complexes. These complexes settle onto the surface of the cultured cells and adhere to the membrane. The cells then internalize the DNA through endocytosis, allowing a fraction of the genetic material to enter the nucleus and be expressed.

**221.** (A) Colony PCR
Colony PCR is the most rapid method for screening large numbers of bacterial colonies to confirm the presence of a recombinant plasmid. A small amount of a colony is added directly to a PCR master mix. During the initial heating step, the bacteria are lysed, releasing the plasmid DNA. The subsequent amplification using insert-specific primers quickly reveals which colonies contain the desired DNA fragment, significantly speeding up the cloning and verification process.

**222.** (A) Knockout
A knockout is an organism that has been genetically engineered to lack the function of a specific gene. This is typically achieved by replacing the endogenous gene with a non-functional or deleted version through targeted homologous recombination. By studying the resulting phenotype of the knockout organism, researchers can determine the natural biological role and importance of the silenced gene, providing essential insights into gene function, development, and the molecular basis of various diseases.

**223.** (A) The microRNA/siRNA regulatory pathway
Experimental RNA interference is a technique that hijacks a cell's natural machinery for gene regulation. In nature, the microRNA and siRNA pathways protect the genome from viruses and regulate endogenous gene expression. By introducing synthetic siRNAs or shRNA vectors, researchers can direct this existing cellular machinery to target and degrade a specific messenger RNA of interest. This results in the temporary or permanent silencing of the target gene, facilitating the study of its function.

**224.** (A) RNA processing and translation
Antisense RNA molecules are designed to be complementary to a specific target messenger RNA. When they hybridize to the mRNA, they form a double-stranded structure that physically obstructs the cellular machinery. This can block the process of splicing during RNA processing or prevent the ribosome from binding to the transcript, thereby inhibiting translation.

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**240.** (A) To generate a set of overlapping fragments ensuring representation of the entire genome
When constructing a genomic library, partial digestion of the DNA is performed to ensure that the entire genome is represented in the library. Complete digestion would cut at every recognition site, potentially splitting genes and creating non-overlapping fragments. Partial digestion, however, generates a diverse set of large, overlapping DNA fragments. This overlap is crucial for physical mapping and for the eventual assembly of the full genome sequence from the individual library clones.

**241.** (A) It requires the DNA solution to have high salt concentrations.
Electroporation is a powerful method for transforming cells, but it is highly sensitive to the ionic strength of the buffer. High salt concentrations in the DNA solution must be strictly avoided because they increase the conductivity of the mixture. This can lead to electrical arcing during the high-voltage pulse, which can destroy both the sample and the equipment. Therefore, DNA for electroporation is typically prepared in water or a very low-salt buffer like ten percent glycerol.

**242.** (A) It emits fluorescence directly upon UV/ blue light excitation without needing exogenous substrates
Green Fluorescent Protein is an exceptionally valuable reporter gene in molecular biology. Unlike other reporters like GUS or luciferase, GFP is autofluorescent, meaning it does not require any exogenous substrates or cofactors to produce a signal. When exposed to blue or ultraviolet light, the protein itself emits bright green fluorescence. This allows for the non-invasive, real-time monitoring of gene expression and protein localization in living cells and whole organisms across diverse species.

**243.** (A) The sequence of the mutagenic oligonucleotide primer
In site-directed mutagenesis using the primer extension method, the location and nature of the mutation are entirely dictated by the synthetic oligonucleotide primer. The researcher designs the primer to be mostly complementary to the target gene but includes one or more mismatched bases at the desired site. During DNA synthesis, the polymerase incorporates these changes into the new strand. This high level of control allows for the precise investigation of specific amino acids in protein function.

**244.** (A) RNA-Induced Silencing Complex
The RNA-induced silencing complex is the central functional unit of the RNA interference pathway. It is a multi-protein assembly that uses a small interfering RNA or microRNA as a guide to identify complementary messenger RNA molecules. Once the target is found, the RISC complex, through its catalytic Argonaute protein, either cleaves the mRNA or prevents its translation. This sophisticated mechanism allows the cell to specifically and effectively silence gene expression at the post-transcriptional level.

**245.** (A) Exceptionally high thermal stability ($T_m$) and nuclease resistance
Locked nucleic acids are a class of high-affinity DNA analogs where the ribose ring is chemically "locked" in a specific conformation. This modification significantly increases the thermal stability of the duplex when an LNA strand binds to its target RNA or DNA. Furthermore, LNAs are highly resistant to degradation by cellular nucleases. These properties make them exceptionally powerful tools for diagnostic assays and for developing therapeutic oligonucleotides that require long-term stability in vivo.

**246.** (A) Continually secrete filamentous phages (like $M_{13}$) without lysing the cell
Phage display technology relies on the unique lifecycle of filamentous bacteriophages like $M_{13}$. Unlike many other viruses, these phages do not kill their host cell. Instead, the infected E. coli continues to grow and divide while constantly secreting new phage particles through the cell membrane. This non-lytic lifestyle allows for the easy collection of large numbers of phages from the culture medium, each displaying a variant protein on its surface for screening.

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**247.** (A) Are produced from a single B-cell clone and recognize a single specific epitope
Monoclonal antibodies are a uniform population of antibodies produced by a single clone of B-lymphocytes. Because they originate from one cell, all the antibody molecules are identical and recognize exactly the same epitope on an antigen. This high specificity and consistency distinguish them from polyclonal antibodies, which are a mixture of different antibodies recognizing multiple epitopes. Monoclonals are

In the laboratory, this enzyme is indispensable for converting messenger RNA into cDNA, which is a prerequisite for molecular cloning, cDNA library construction, and measuring gene expression through techniques like RT-PCR and RNA sequencing.

**279.** (A) Strong promoter
A standard cloning vector for use in E. coli must contain several essential elements to be functional. These include an origin of replication for autonomous maintenance, a selectable marker to identify transformed cells, and a multiple cloning site for inserting DNA. However, if the goal is simply to clone and propagate the DNA rather than express a protein, a strong promoter is not required. Promoters are only necessary in expression vectors to drive the transcription of the inserted gene.

**280.** (A) Adapters provide cohesive ends, which ligate with significantly higher efficiency
When constructing a cDNA library, researchers often use adaptors rather than blunt-end ligation to join the cDNA to the vector. Adaptors are synthetic oligonucleotides that provide preformed cohesive, or sticky, ends. The ligation of complementary sticky ends is significantly more efficient than blunt-end ligation because the overhangs help to align and stabilize the two DNA molecules, making it much easier for the DNA ligase to form the phosphodiester bonds. This significantly increases the total number of recombinant clones.

**281.** (A) A defective beta-galactosidase encoded by the host and a small peptide encoded by the plasmid
Alpha-complementation is a phenomenon where two non-functional fragments of the beta-galactosidase enzyme come together to form an active enzyme. The host bacterial cell carries a mutation that produces a defective enzyme lacking the amino-terminal alpha peptide. The plasmid vector carries a small DNA sequence that encodes this missing alpha peptide. When both are present in the same cell, they physically interact to restore enzymatic activity, allowing for the colorimetric identification of recombinant colonies during blue-white screening.

**282.** (A) Methylated DNA (specifically GmATC sequences)
In site-directed mutagenesis protocols, the restriction enzyme DpnI is used to selectively eliminate the non-mutated parental DNA template after the PCR reaction. DpnI is unique because it only cleaves DNA that is methylated at its target sequence. Since the parental plasmid was isolated from a methylated bacterial source and the newly synthesized mutant DNA is produced in vitro and remains unmethylated, DpnI treatment specifically destroys the wild-type template. This ensuring that only the desired mutant plasmids are transformed into host cells.

**283.** (A) An interferon response leading to global translation shutdown
In mammalian cells, introducing long double-stranded RNA molecules larger than thirty base pairs typically triggers a powerful innate immune response known as the interferon response. This pathway evolved to protect cells from viral infections. When long dsRNA is detected, it leads to the global shutdown of all protein synthesis and the non-specific degradation of cellular RNA, often resulting in cell death. To achieve specific gene silencing without this toxicity, researchers use small interfering RNAs which are too short to trigger the interferon pathway.

**284.** (A) Is identical to human insulin, thereby eliminating allergic reactions
Recombinant human insulin is superior to traditional animal-derived versions because its amino acid sequence is identical to the insulin naturally produced by the human body. Before recombinant technology, insulin was purified from the pancreases of cows or pigs. While effective, these animal insulins differ slightly in sequence and can cause allergic reactions or immune responses in some diabetic patients over long-term use. The recombinant version, produced in E. coli or yeast, eliminated these issues, providing a safer and more consistent treatment.

**285.** (A) Maintaining adequate aeration, mixing, and heat transfer
Scaling up a fermentation process from small laboratory flasks to large industrial bioreactors is a significant engineering challenge. As the volume increases, it becomes much more difficult to ensure that all the microorganisms receive

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Unlike the Sanger method, which uses chain termination, Maxam-Gilbert relies on specific chemical modifications followed by the cleavage of the DNA backbone at the modified sites. By using different chemical treatments to target each of the four bases separately, researchers can generate a series of fragments that, when resolved by gel electrophoresis, reveal the sequence of the DNA molecule based on their lengths.

**256.** (A) $SP_6$ or $T_7$ RNA polymerase
In vitro transcription is a powerful method for generating large amounts of specific RNA transcripts from a DNA template. The reaction utilizes a highly efficient RNA polymerase, most commonly derived from bacteriophages like $T_7$ or $SP_6$. These enzymes recognize their specific promoters with high fidelity. By cloning a target gene downstream of such a promoter, researchers can use these polymerases to synthesize pure RNA for use as probes, in translation studies, or for RNA interference experiments.

**257.** (A) An N-terminal signal sequence targeting the Sec or Tat pathway
To achieve the secretion of a recombinant protein in E. coli, the protein must be directed across the inner membrane. This is accomplished by fusing an N-terminal signal sequence to the protein, which is recognized by the cell's secretory machinery, such as the Sec or Tat pathways. Secretion into the periplasm or the culture medium offers several advantages, including proper disulfide bond formation, reduced protein aggregation, and a significantly simpler purification process compared to intracellular expression.

**258.** (A) Physical sizing selection
The in vitro packaging of bacteriophage lambda DNA serves as a natural selection mechanism during cloning. The packaging machinery only accepts DNA molecules that fall within a specific size range, approximately seventy-eight to one hundred and five percent of the wild-type genome. This means that only recombinant molecules that have successfully incorporated an insert of the correct size will be viable. This physical sizing selection ensures that the resulting library contains a high proportion of clones with significant inserts.

**259.** (A) DNA
Southern blotting is a classic molecular biology technique used to detect the presence and size of specific DNA sequences within a complex mixture. It involves several key steps: digesting DNA with restriction enzymes, separating the fragments by gel electrophoresis, and then transferring the separated DNA molecules from the gel onto a solid membrane, such as nitrocellulose. The specific DNA of interest is then identified by hybridizing the membrane with a labeled nucleic acid probe that is complementary to the target.

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**260.** (A) Introduce a specific mutation or a reporter gene precisely at the endogenous locus
A gene knockin is a targeted genetic engineering technique used to insert a specific DNA sequence into a precise, predetermined location in the genome. Unlike transgenic methods that result in random integration, a knockin uses homologous recombination to place the new sequence at the endogenous locus of a gene. This is often used to introduce specific mutations to model diseases or to insert a reporter gene that will be regulated by the gene's natural promoter.

**261.** (A) 3' Untranslated Region (3' UTR)
MicroRNAs are small, non-coding RNAs that play a central role in post-transcriptional gene regulation. They typically exert their effects by binding to specific target sites within the 3' untranslated region of messenger RNA molecules. This binding is mediated by the "seed sequence" at the 5' end of the miRNA. Depending on the degree of complementarity, the miRNA either triggers the degradation of the mRNA or inhibits its translation, thereby reducing the amount of protein produced by the cell.

**262.** (A) Dissolve blood clots in patients with stroke or myocardial infarction
Tissue plasminogen activator is a crucial recombinant therapeutic protein that functions as a powerful thrombolytic agent. It works by activating plasminogen into plasmin, which then breaks down fibrin, the primary component of blood clots. In clinical practice, tPA is administered to patients suffering from acute ischemic stroke or myocardial infarction to dissolve the obstructive clots and restore blood flow to the affected organs. The development of recombinant tPA has significantly improved the emergency treatment of these conditions.

**263.** (A) Vitamin A
Golden Rice is a genetically modified variety of rice engineered to biosynthesize beta-carotene, a

precursor of Vitamin A, in the edible part of the grain. The crop was developed as a humanitarian tool to address Vitamin A deficiency, which is a leading cause of blindness and death in many developing countries where rice is a dietary staple. By providing a common source of provitamin A, Golden Rice aims to improve public health and reduce the prevalence of nutritional blindness.

**264.** (A) Permit someone else to produce the patented product without the consent of the patent owner under specific circumstances
Compulsory licensing is a provision under the TRIPS Agreement that allows a government to grant permission to a third party to produce a patented invention without the consent of the patent holder. This typically occurs during public health emergencies or when the patented product is not accessible at a fair price. While it protects public interests, the government must still ensure that the patent owner receives adequate compensation. This mechanism balances intellectual property rights with the need for affordable medicine.

**265.** (A) It reads the same 5' to 3' on both complementary strands
In molecular biology, a DNA sequence is considered palindromic if its 5' to 3' sequence is identical on both the sense and the complementary antisense strands. This type of rotational symmetry is a hallmark of the recognition sites for many Type II restriction endonucleases. Because both strands present the same chemical signature to the enzyme, it can bind as a homodimer and cleave each strand at a specific, symmetrical position, resulting in predictable DNA fragments for cloning.

**266.** (A) 3' to 5' proofreading exonuclease activity
Taq DNA polymerase is a highly efficient, thermostable enzyme essential for PCR, but it lacks the 3' to 5' proofreading exonuclease activity found in many other polymerases. This means the enzyme cannot correct mismatched nucleotides during DNA synthesis. As a result, Taq has a relatively high error rate, approximately one mutation per few thousand base pairs. For experiments where absolute sequence fidelity is critical, such as cloning or high-accuracy sequencing, other proofreading-capable polymerases are typically used.

**267.** (A) Provide a cluster of unique restriction enzyme recognition sites for insert insertion
The multiple cloning site is a short, engineered DNA sequence within a plasmid vector that contains a cluster of several unique restriction enzyme recognition sites. These sites are strategically chosen to appear only once in the entire vector, providing a versatile entry point for foreign DNA fragments. The MCS allows researchers to use different enzymes for cloning, facilitating the directional insertion of genes and making the vector compatible with a wide range of restriction digestion strategies.

**268.** (A) Electrophoretic Mobility Shift Assay (EMSA)
The Electrophoretic Mobility Shift Assay is a powerful technique used to study the interaction between proteins and specific DNA sequences. The assay is based on the principle that a protein-DNA complex will migrate more slowly through a non-denaturing gel than free DNA. By comparing the migration of labeled DNA in the presence and absence of a protein, researchers can determine if a protein binds to the sequence, providing essential insights into gene regulation and transcription factor binding.

**269.** (A) Crown gall disease
The Ti plasmid, naturally carried by Agrobacterium tumefaciens, is the causative agent of crown gall disease in many dicotyledonous plants. The bacterium infects wounded plant tissues and transfers a specific segment of the plasmid, the T-DNA, into the host cell's genome. The T-DNA contains genes that trigger the overproduction of plant hormones, leading to uncontrolled cell division and the formation of characteristic tumors, or galls, at the site of infection, usually near the soil line.

**270.** (A) RNA viruses
RNA interference is a natural biological mechanism that is thought to have evolved in plants and other organisms as a defense against viral infections. Many plant viruses carry their genetic information as RNA and produce double-stranded RNA intermediates during replication. The RNAi machinery recognizes these foreign dsRNA molecules and targets them for degradation, effectively silencing the virus. This evolutionary adaptation provides a robust and highly specific immune response that helps plants resist a wide range of viral pathogens.

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**271.** (A) Complementarity Determining Regions (CDRs) in the variable domains
The remarkable specificity of an antibody for its target antigen is determined by its complementarity determining regions. These are six hypervariable loops, three on each of the heavy and light chain variable domains, that form the actual antigen-binding site. The unique amino acid sequence and shape of these CDRs allow the antibody to bind to a specific epitope on an antigen with high affinity. Humanization techniques focus on preserving these mouse-derived loops while replacing the rest of the antibody.

**272.** (A) Both the personnel and the product
A Class II biosafety cabinet is a specialized laboratory enclosure designed to provide a sterile environment for research. It utilizes a precise laminar airflow and HEPA filtration to protect three elements: the personnel, by creating an inward air curtain; the product or experiment, by providing clean, filtered air over the work surface; and the environment, by filtering all exhaust air. This balanced protection makes Class II cabinets essential for working with infectious agents and maintaining sterile cell cultures.

**273.** (A) Not be obvious to a person having ordinary skill in the art
The inventive step is a fundamental requirement for the patentability of an invention. It means that the development must not be something that would have been obvious to a person with ordinary skill in that specific technical field at the time the application was filed. This criteria ensures that patents are only granted for truly creative and significant contributions to technology, rather than for simple or logical improvements that anyone in the field could have easily conceived.

**274.** (A) Differences in solubility of molecules between aqueous and organic phases
Organic extraction of nucleic acids is based on the differential solubility of cellular components in aqueous and organic solvents. When a cell lysate is mixed with phenol and chloroform, the non-polar lipids and denatured proteins migrate into the lower organic phase or accumulate at the interphase. The highly polar DNA and RNA remain dissolved in the upper aqueous phase. This phase separation allows for the clean recovery of purified nucleic acids by simply collecting the top layer after centrifugation.

**275.** (A) Gamma
$T_4$ polynucleotide kinase is an enzyme that catalyzes the phosphorylation of the 5'-hydroxyl ends of DNA or RNA. The enzyme utilizes adenosine triphosphate as the phosphate donor. Specifically, it transfers the terminal gamma-phosphate of the ATP molecule to the 5'-OH group of the nucleic acid substrate. This reaction is widely used in molecular biology for radiolabeling DNA probes and for preparing DNA fragments with the necessary 5' phosphate for subsequent ligation reactions by DNA ligase.

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**276.** (A) Incubation at $T_m - 25^\circ C$ with moderate to high salt concentration
For denatured DNA strands to re-anneal, or renature, the conditions must be optimized to favor stable base pairing. The most effective temperature for renaturation is typically about twenty-five degrees below the melting temperature of the DNA. Additionally, a moderate to high salt concentration is required to neutralize the electrostatic repulsion between the negatively charged phosphate backbones of the two strands. These conditions provide enough energy for random collisions while ensuring that only perfectly complementary sequences form stable double helices.

**277.** (A) The ratio and concentration of acrylamide to bisacrylamide
The resolving power of a polyacrylamide gel is primarily determined by its pore size, which is controlled by the total concentration of acrylamide and the amount of the cross-linker bisacrylamide. By adjusting this ratio, researchers can tailor the gel to separate molecules within a specific size range. A higher concentration of acrylamide results in smaller pores, which is ideal for resolving very small DNA fragments or proteins with small differences in molecular weight, providing superior resolution compared to agarose gels.

**278.** (A) RNA-dependent DNA polymerase
Reverse transcriptase is a specialized enzyme that functions as an RNA-dependent DNA polymerase. It was originally discovered in retroviruses, where it is used to copy the viral RNA genome into a complementary DNA strand that can be integrated into the host cell's DNA.

essential tools in diagnostics, research, and as highly targeted therapeutic agents for many diseases.

**248.** (A) Perform sophisticated post-translational modifications like glycosylation
While E. coli is a highly efficient host for producing many simple proteins, it has significant limitations for complex human therapeutics. As a prokaryote, it lacks the specialized organelles, like the endoplasmic reticulum and Golgi apparatus, required for eukaryotic post-translational modifications. Most importantly, it cannot perform glycosylation, the addition of complex sugar chains that is often essential for the proper folding, stability, and biological activity of many human proteins, such as erythropoietin or various antibodies.

**249.** (A) Rapid clearance and degradation by serum nucleases
Delivering naked plasmid DNA into the body for gene therapy is generally unsuccessful because the bloodstream is a hostile environment for nucleic acids. The blood contains high levels of nuclease enzymes that rapidly degrade the DNA into small, inactive fragments. Additionally, naked DNA is quickly cleared from the circulation by the kidneys or captured by the liver. To be effective, therapeutic DNA must be protected within a delivery vehicle, such as a viral vector or lipid nanoparticle.

**250.** (A) A strong, constitutive promoter (e.g., CaMV 35S)
In plant transformation, it is essential that the selectable marker gene is expressed consistently across all tissues to ensure that only transgenic cells survive the selection process. Therefore, these genes are typically placed under the control of a strong, constitutive promoter. The cauliflower mosaic virus 35S promoter is the most common choice, as it provides high levels of gene expression throughout the entire plant, allowing for the reliable identification and regeneration of transgenic plant lines.

**251.** (A) Concerns from the public and scientists regarding comprehensive safety testing and biodiversity impact
The environmental release of Bt Brinjal in India was met with significant public and scientific debate. While the Genetic Engineering Appraisal Committee initially recommended its commercialization, the government later imposed an indefinite moratorium. This decision was driven by widespread concerns regarding the adequacy of safety testing, the potential for gene flow to wild relatives, and the impact on biodiversity. The case highlights the complex social, ethical, and scientific challenges involved in the regulation of genetically modified food crops.

**252.** (A) All information that has been disclosed to the public in any form before a given date
In patent law, prior art is a critical concept used to determine the novelty and non-obviousness of an invention. It encompasses all information that was available to the public in any form, including previously published patents, scientific papers, or even public demonstrations, anywhere in the world before the filing date of the patent application. If an invention is already described in the prior art, it cannot be considered new, and a patent will not be granted.

**253.** (A) DNA binds to silica in the presence of high concentrations of chaotropic salts
The purification of DNA using silica-based columns relies on a unique chemical interaction. In the presence of high concentrations of chaotropic salts, such as guanidinium thiocyanate, the hydration shell of the DNA molecule is disrupted, allowing it to bind specifically to the silica matrix. Other cellular components, such as proteins and carbohydrates, do not bind and are washed away. The purified DNA is then released from the silica by switching to a low-salt buffer or pure water.

**254.** (A) Presence of one or two G or C bases at the 3' end to promote specific binding
When designing primers for PCR, including a GC clamp at the 3' end is a standard recommendation. This involves ensuring that the last one or two bases of the primer are either guanine or cytosine. Because G and C bases form three hydrogen bonds, they bind more strongly than adenine or thymine. This "clamp" ensures that the 3' end of the primer hybridizes tightly to the template, facilitating efficient and specific extension by the DNA polymerase.

**255.** (A) Chemical modification and specific cleavage of DNA bases
The Maxam-Gilbert method was one of the first techniques developed for DNA sequencing.

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sufficient oxygen and nutrients. Problems with mixing can lead to localized nutrient depletion or the buildup of metabolic waste. Additionally, large-scale cultures generate significant heat that must be efficiently removed to maintain the optimal temperature for the cells. Successfully managing these parameters is critical for maintaining high productivity and product quality.

**286.** (A) Coating them with polyethylene glycol (PEG)
Liposomes used for the systemic delivery of drugs or vaccines are often cleared rapidly from the bloodstream by the immune system's macrophages. This clearance can be significantly reduced by coating the surface of the liposomes with polyethylene glycol. This process, known as PEGylation, creates a protective hydrated layer that shields the liposomes from detection by the reticuloendothelial system. By becoming "stealthy," these modified liposomes can circulate for much longer periods, allowing for more effective accumulation at the intended therapeutic target site.

**287.** (A) A Type IV secretion system that transfers the T-DNA complex
Agrobacterium tumefanciens uses a highly specialized mechanism to transfer its genetic material into plant cells. This process is mediated by a Type IV secretion system, a complex protein apparatus that spans the bacterial cell membrane. When the bacterium detects signals from a wounded plant, it uses this system to actively transport a single-stranded T-DNA molecule, along with several virulence proteins, into the plant cytoplasm and subsequently into the nucleus, where it integrates into the host genome.

**288.** (A) GEAC
In the Indian regulatory system, the Genetic Engineering Appraisal Committee is the ultimate authority for approving the commercial release of genetically modified crops. Before a GMO can be grown by farmers or sold in the market, it must undergo extensive safety and environmental evaluations by the GEAC. The committee reviews all the data from field trials and laboratory studies to ensure that the transgenic crop does not pose any significant risk to human health, animal welfare, or the environment in India.

**289.** (A) The unauthorized use or patenting of biological resources and traditional knowledge without fair compensation
Biopiracy refers to the practice where corporations or researchers from developed countries exploit biological resources or traditional knowledge from indigenous communities in developing nations. This often involves filing patents on products derived from these resources, such as medicinal plants, without providing any fair compensation or acknowledging the original knowledge holders. International agreements like the Convention on Biological Diversity aim to prevent biopiracy by ensuring that the benefits of biological resources are shared equitably with the countries and communities of origin.

**290.** (A) RT-PCR
Reverse transcription-polymerase chain reaction is a widely used technique that allows for the detection and quantification of RNA. The process begins with the enzyme reverse transcriptase, which uses the RNA sample as a template to synthesize a complementary DNA molecule. This cDNA then serves as the template for a standard PCR amplification. RT-PCR is an essential tool for studying gene expression, cloning genes from mRNA, and detecting the presence of RNA viruses in clinical and environmental samples.

**291.** (A) To lower the melting temperature of double-stranded nucleic acids
Formamide is a chemical agent commonly added to nucleic acid hybridization buffers. Its primary role is to lower the melting temperature of the DNA or RNA duplexes by disrupting the hydrogen bonds between the complementary base pairs. By reducing the $T_m$, formamide allows researchers to conduct hybridization and washing steps at lower temperatures. This is important for maintaining the structural integrity of the membrane and the nucleic acids while still ensuring that the hybridization occurs with high sequence specificity.

**292.** (A) It catalyzes the insertion of the viral DNA into the host genome
Integrase is a critical enzyme encoded by retroviruses, such as HIV, and utilized in retroviral vector systems. Its primary function is to catalyze the covalent insertion of the viral DNA, which has been reverse-transcribed from RNA, into the genomic DNA of the host cell. This integration is essential for the stable

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# CHAPTER 05
# Cell Culture and Hybridoma Technology

2015
**1.** Who pioneered the first successful animal cell culture using frog nerve fibers?
(A) Alexis Carrel
(B) Harry Eagle
(C) George Gey
(D) Ross Harrison

2024
**2.** What major milestone was achieved by George Gey in 1951?
(A) Discovery of HAT medium
(B) Formulation of MEM
(C) Establishment of the HeLa cellline
(D) Development of hybridoma technology

2018
**3.** Which researchers developed hybridoma technology for monoclonal antibody production?
(A) Watson and Crick
(B) Kohler and Milstein
(C) Eagle and Dulbecco
(D) Carrel and Harrison

2005
**4.** Identify the INCORRECT statement regarding Alexis Carrel's contribution.
(A) He developed the flask technique
(B) He maintained chick embryo heart cells for decades
(C) He strictly emphasized aseptic techniques
(D) He invented the first chemically defined medium

2002
**5.** Harry Eagle is primarily recognized for developing which crucial culture component?
(A) Basal growth media
(B) Fetal bovine serum
(C) Trypsinization protocols
(D) Hollow-fiber bioreactors

2018
**6.** Which discovery allowed the elimination of feeder layers in many cell cultures?
(A) Use of antibiotics
(B) Addition of continuous perfusion
(C) Chemically defined growth factors
(D) Discovery of somatic cell fusion

2025
**7.** The introduction of laminar flow biological safety cabinets drastically improved which process?
(A) Aseptic cell manipulation
(B) Cellular immortalization
(C) Antibody humanization
(D) Karyotype analysis

2024
**8.** The first commercially produced recombinant protein from mammalian cells was:
(A) Murine monoclonal antibody
(B) Human growth hormone
(C) Biosynthetic human insulin
(D) Tissue plasminogen activator

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2001
**9.** Identify the correct pair regarding cell culture milestones.
(A) Dulbecco - Trypsinization
(B) Enders - Polio virus in culture
(C) Earle - Hybridoma technology
(D) Sato - HeLa cell line

2013
**10.** Consider the following statements regarding early cell culture discoveries:
I. Earle established the L cell line
II. Rous isolated the first tumor-causing virus
III. Eagle developed monoclonal antibodies
Which of the above statement(s) is/are correct?
(A) I and II
(B) I and III
(C) II only
(D) I, II and III

2002
**11.** Which method is most appropriate for sterilizing heat-labile culture media?
(A) Autoclaving
(B) Dry heat sterilization
(C) UV irradiation
(D) Membrane filtration

2017
**12.** What is the standard pore size used for filter-sterilizing culture media to remove bacteria?

**300.** (A) Both A and R are true and R is the correct explanation of A.
The patenting of human genes is a major ethical issue in biotechnology. The primary argument against such patents is that genes are naturally occurring biological entities rather than human inventions. Granting exclusive rights to a specific gene sequence could hinder medical progress by limiting other researchers' access to that gene for study or diagnostic testing. Therefore, many argue that natural genetic information should remain in the public domain, while only the novel applications or synthetic modifications of that information should be eligible for patent protection.

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maintenance and long-term expression of the viral or recombinant genes. Because the integration is permanent, it ensures that the genetic material is passed on to all daughter cells.

**293.** (A) Lysozyme
The first step in isolating DNA from bacterial cells is to break down the protective cell wall. For many bacteria, this is achieved using the enzyme lysozyme. Lysozyme specifically catalyzes the hydrolysis of the beta-glycosidic bonds in the peptidoglycan layer of the cell wall, weakening the structure and causing the cells to burst, or lyse, when exposed to detergents or osmotic stress. This enzymatic digestion is essential for releasing the genomic and plasmid DNA into the lysis buffer for subsequent purification.

**294.** (A) They assemble mature phage particles around the recombinant cosmid or lambda DNA
Bacteriophage packaging extracts are essential tools for constructing genomic libraries in lambda or cosmid vectors. These extracts consist of pre-assembled empty phage heads and tail proteins. When mixed with recombinant DNA that contains the proper cos sites, the extracts automatically package the DNA into the phage heads and attach the tails. This in vitro process creates functional, infectious phage particles that can deliver the recombinant DNA into E. coli with much higher efficiency than traditional chemical transformation methods.

**295.** (A) Immunological screening (e.g., Western blot/ colony immunoblot)
Immunological screening is a technique used to identify specific clones in an expression library based on the protein they produce. Recombinant bacterial colonies are transferred to a membrane and lysed to release their proteins. The membrane is then incubated with a primary antibody that specifically recognizes the protein of interest. A secondary antibody conjugated to an enzyme then allows for the visual detection of the specific colony that is expressing the target gene, facilitating the isolation of the correct clone.

**296.** (A) Highly specific, programmable, and efficient targeted DNA double-strand breaks
The CRISPR-Cas9 system has transformed reverse genetics by providing a precise and programmable tool for genome editing. It uses a guide RNA to direct the Cas9 nuclease to a specific DNA sequence, where the enzyme creates a double-strand break. The cell's natural repair mechanisms then fix the break, often introducing mutations that knockout the gene or allowing for the precise insertion of new DNA. This technology makes it much easier and faster to study gene function in diverse organisms.

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**297.** (A) Precisely in the middle of the region paired with the siRNA guide strand
In the RNA interference pathway, the Argonaute protein within the RISC complex uses the small interfering RNA guide strand to identify complementary messenger RNA. Once the target is correctly paired, the Argonaute enzyme cleaves the mRNA backbone at a very specific location. This cleavage typically occurs exactly in the middle of the region where the guide strand is hybridized, usually between the tenth and eleventh nucleotides. This precise cut leads to the rapid degradation of the transcript and the silencing of the gene.

**298.** (A) Their capsids can accommodate variable lengths of single-stranded DNA and surface fusion proteins
Filamentous bacteriophages like $M_{13}$ are uniquely suitable for phage display because their flexible, rod-shaped capsid can be easily extended to accommodate large inserts of DNA. This allows researchers to fuse a wide variety of proteins or peptides to the phage coat proteins without interfering with the assembly of the virus. Because the phage is secreted without killing the host cell, it provides a stable and versatile platform for displaying and screening vast libraries of protein variants for specific binding interactions.

**299.** (A) It allows for the visual tracking of gene expression spatial patterns in tissues
The GUS reporter gene system is an invaluable tool for studying gene regulation in transgenic plants. When the uidA gene is expressed under a specific promoter, it produces the beta-glucuronidase enzyme. By providing the substrate X-gluc, which is converted into a blue precipitate, researchers can visually map the exact locations where the promoter is active within the plant tissues. This allows for the precise tracking of gene expression patterns across different organs, developmental stages, and in response to various environmental stimuli.

(A) 0.22 μm
(B) 0.45 μm
(C) 1.20 μm
(D) 5.00 μm

2023
**13.** At what temperature and pressure is routine autoclaving performed?
(A) 100°C at 10 psi
(B) 110°C at 12 psi
(C) 121°C at 15 psi
(D) 130°C at 20 psi

2010
**14.** Which equipment is best sterilized using dry heat?
(A) Plastic flasks
(B) Glassware
(C) Liquid media
(D) Growth factors

2006
**15.** The sterilization mechanism of an autoclave relies primarily on:
(A) Protein coagulation via moist heat
(B) Oxidative damage
(C) DNA cross-linking
(D) Membrane disruption by pressure

2024
**16.** UV irradiation in biological safety cabinets sterilizes through which mechanism?
(A) Denaturing proteins
(B) Producing high heat
(C) Forming thymine dimers in DNA
(D) Generating strong alkaline radicals

2025
**17.** Which sterilization method causes the least structural damage to empty plastic petri dishes?
(A) Autoclaving
(B) Dry heat
(C) Boiling
(D) Gamma irradiation

2018
**18.** Why is filtration preferred over heat for sterilizing fetal bovine serum?
(A) It destroys mycoplasma completely
(B) Filtration increases serum osmolarity
(C) Heat degrades essential growth factors
(D) Filters bind toxic heavy metals

2021
**19.** Identify the INCORRECT pair of sterilization targets and methods.
(A) Metal surgical tools - Autoclave
(B) Liquid antibiotics - Autoclave
(C) Empty glassware - Dry heat oven
(D) Air in safety cabinet - HEPA filter

2010
**20.** Consider the following statements about sterilization:
I. HEPA filters remove 99.97% of 0.3 μm particles
II. Autoclaving requires 15 minutes at 121°C
III. Ethylene oxide is used for liquid media
IV. Dry heat requires higher temperatures than moist heat
Which of the above statement(s) is/are INCORRECT?
(A) I, II and III
(B) III only
(C) I and IV
(D) II, III and IV

2003
**21.** What is the optimal pH range for most mammalian cell cultures?
(A) 6.8 - 7.0
(B) 7.2 - 7.4
(C) 7.6 - 7.8
(D) 8.0 - 8.2

2008
**22.** Which buffering system is most commonly used in cell culture incubators?
(A) Phosphate-buffered saline
(B) Tris-HCl
(C) Bicarbonate-carbon dioxide
(D) HEPES

2024
**23.** Teleost fish cell cultures typically require which temperature range?
(A) 4 - 10°C
(B) 15 - 25°C
(C) 30 - 35°C
(D) 37 - 40°C

2024
**24.** What is the standard osmolarity range for mammalian cell culture media?
(A) 100 - 150 mOsm/kg
(B) 280 - 320 mOsm/kg
(C) 400 - 450 mOsm/kg
(D) 500 - 600 mOsm/kg

2013
**25.** Phenol red in culture media acts primarily as a:
(A) Growth stimulant
(B) pH indicator

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2011

**101.** Which large-scale culture system uses gas sparging for mixing rather than a mechanical impeller?
(A) Airlift bioreactor
(B) Stirred-tank bioreactor
(C) Hollow-fiber system
(D) Cell factory

2011

**102.** In a stirred-tank bioreactor, what prevents a deep vortex and improves mixing efficiency?
(A) Impeller speed reduction
(B) Sparger rings
(C) Baffles
(D) Microcarriers

2001

**103.** Scale-up of anchorage-dependent cells most commonly utilizes:
(A) High-speed centrifugation
(B) Deep-well plates
(C) Airlift fermenters
(D) Microcarriers

2009

**104.** Which parameter is highly critical and constantly monitored during large-scale bioreactor runs?
(A) DNA content
(B) Intracellular lipid levels
(C) Dissolved oxygen (DO)
(D) Telomere length

2007

**105.** Airlift bioreactors are generally preferred over stirred-tank bioreactors for animal cells because they:
(A) Generate higher shear stress
(B) Generate lower shear stress, protecting fragile cells
(C) Require no aeration
(D) Can only be used for bacterial cells

2013

**106.** A common foaming issue in sparged bioreactors is mitigated by adding:
(A) Extra serum
(B) Trypsin
(C) High concentrations of glucose
(D) Pluronic F-68 or anti-foaming agents

2017

**107.** The height-to-diameter ratio in an airlift bioreactor is typically:
(A) 0.5:1
(B) 1:1
(C) 2:1
(D) 10:1 or greater

2001

**108.** Which mode of bioreactor operation involves continuous addition of fresh medium and simultaneous removal of spent medium containing cells?
(A) Continuous culture (Chemostat)
(B) Batch culture
(C) Fed-batch culture
(D) Static culture

2006

**109.** Fed-batch culture is advantageous for monoclonal antibody production because it:
(A) Keeps cells in the lag phase indefinitely
(B) Completely prevents toxic metabolite formation
(C) Prolongs the productive stationary/decline phase
(D) Requires no environmental monitoring

2008

**110.** Consider the following statements regarding bioreactors:
I. Stirred tanks rely on impellers
II. Airlift systems utilize gas bubbles for circulation
III. Animal cells tolerate high shear stress better than bacteria
Which of the above statement(s) is/are correct?
(A) I and II
(B) I and III
(C) II and III
(D) I only

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2010

**111.** Microcarriers are small beads typically made of:
(A) Solid stainless steel
(B) Dextran, gelatin, or glass
(C) Pure lipids
(D) Teflon

2009

**112.** The specific advantage of using microcarriers in scale-up is:
(A) They convert adherent cells to suspension
(B) They eliminate the need for liquid media
(C) They synthesize essential growth factors
(D) They provide high surface area to volume ratios

2013

**113.** In a hollow-fiber bioreactor system, cells usually grow:
(A) In the extracapillary space surrounding the

2009

**39.** Which vitamin is a key constituent of standard basal media?
(A) Thiamine
(B) Ascorbic acid
(C) Vitamin K
(D) Vitamin D

2018

**40.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** L-glutamine must be supplemented frequently in prolonged cell cultures.
**Reason (R):** L-glutamine spontaneously degrades into ammonia, which is toxic to cells.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2015

**41.** What is the primary role of Fetal Bovine Serum (FBS) in cell culture?
(A) To provide an energy source
(B) To supply growth factors and hormones
(C) To maintain medium pH
(D) To act as a pH indicator

2019

**42.** Which serum protein functions to bind and neutralize heavy metals and toxins?
(A) Fibronectin
(B) Transferrin
(C) Albumin
(D) Macroglobulin

2007

**43.** Why is heat inactivation (56°C for 30 mins) often performed on serum?
(A) To denature high-weight albumin
(B) To activate latent growth factors
(C) To sterilize against heat-stable bacteria
(D) To destroy complement system proteins

2001

**44.** Which attachment factor is abundantly present in FBS?
(A) Fibronectin
(B) Insulin
(C) Transferrin
(D) Selenium

2024

**45.** A major disadvantage of using undefined serum in cell culture is:
(A) Lack of attachment factors
(B) Batch-to-batch variability
(C) Excessive buffering capacity
(D) Depletion of amino acids

2014

**46.** What is the typical concentration range of serum used in routine mammalian cell culture?
(A) 1 - 2%
(B) 5 - 10%
(C) 20 - 30%
(D) 40 - 50%

2016

**47.** Chemically defined media must often be supplemented with ITS. What does ITS stand for?
(A) Insulin, Transferrin, Selenium
(B) Isoleucine, Threonine, Serine
(C) Iron, Tyrosine, Sodium
(D) Inositol, Thymidine, Sphingomyelin

2007

**48.** In serum-free media, what is the specific role of transferrin?
(A) Facilitates glucose uptake
(B) Promotes cellular adhesion
(C) Mediates iron transport
(D) Acts as a protease inhibitor

2018

**49.** Which of the following is a direct advantage of serum-free media?
(A) Universal applicability to all cell types
(B) Lower cost compared to basal media
(C) Requires less strict aseptic technique
(D) Easier downstream purification of products

2002

**50.** Match the culture supplement in List I with its primary function in List II.
List I: (Supplement)
P. Insulin
Q. Fibronectin
R. Ethanolamine
S. Selenium
List II: (Function)
1. Cell attachment
2. Phospholipid synthesis
3. Glucose uptake
4. Antioxidant enzyme cofactor
(A) P-3, Q-1, R-2, S-4
(B) P-1, Q-3, R-4, S-2

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(C) P-3, Q-2, R-1, S-4
(D) P-2, Q-1, R-3, S-4

2016
**51.** The process of isolating cells directly from tissue to establish a culture is called:
(A) Subculturing
(B) Primary culture
(C) Continuous culture
(D) Transformation

2024
**52.** Which enzyme is most widely used for the dissociation of tissues into single cells?
(A) Ligase
(B) Amylase
(C) Polymerase
(D) Trypsin

2018
**53.** Mechanical disruption of tissue is most suitable for which type of tissue?
(A) Soft tissues like spleen or brain
(B) Hard bone tissue
(C) Dense fibrous connective tissue
(D) Cartilage

2025
**54.** Collagenase is particularly preferred over trypsin for dissociating which tissues?
(A) Liquid tissues like blood
(B) Embryonic brain tissue
(C) Fibrous and connective tissues
(D) Cultured continuous cell lines

2022
**55.** What is the purpose of adding EDTA during enzymatic tissue dissociation?
(A) To chelate calcium and magnesium, weakening cell junctions
(B) To provide nutrients to dissociated cells
(C) To inhibit bacterial contamination
(D) To neutralize trypsin activity

2002
**56.** The explant culture method relies primarily on which cellular behavior?
(A) Induced cellular transformation
(B) Active cellular phagocytosis
(C) Total anchorage independence
(D) Outgrowth and migration from the tissue

2018
**57.** Which primary culture technique yields the highest initial viable cell number?
(A) Explant culture
(B) Warm enzymatic dissociation
(C) Mechanical mincing without enzymes
(D) Cold trypsinization

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2011
**58.** Cold trypsinization is often preferred to warm trypsinization because it:
(A) Is significantly faster to perform
(B) Requires no subsequent centrifugation
(C) Minimizes damage and preserves viability
(D) Inactivates bovine serum proteins

2011
**59.** Identify the correct statement about primary cultures.
(A) They possess an infinite lifespan in vitro
(B) They are inherently and strictly tumorigenic
(C) They consist of one cell type upon isolation
(D) They mimic the in vivo tissue characteristics

2002
**60.** Select the correct sequence of steps for enzymatic tissue dissociation.
(A) Mincing, Washing, Enzyme incubation, Centrifugation, Resuspension
(B) Enzyme incubation, Mincing, Centrifugation, Washing, Resuspension
(C) Washing, Centrifugation, Mincing, Enzyme incubation, Resuspension
(D) Resuspension, Mincing, Enzyme incubation, Centrifugation, Washing

2011
**61.** Normal primary somatic cells undergo a limited number of divisions in vitro. This is called the:
(A) Restriction point
(B) Hayflick limit
(C) Plateau limit
(D) Senescence barrier

2014
**62.** Which phase of the cell growth curve exhibits exponential cell proliferation?
(A) Lag phase
(B) Log phase
(C) Stationary phase
(D) Death phase

2018
**63.** Contact inhibition in normal cultured cells causes them to:
(A) Stop dividing when they reach confluency
(B) Grow over each other in multiple layers
(C) Differentiate into fibroblasts
(D) Undergo immediate apoptosis

2011

**64.** The lag phase of a growth curve is characterized by:
(A) Rapid DNA replication
(B) Depletion of media nutrients
(C) Cell attachment and adaptation without division
(D) Accumulation of toxic metabolites

2016

**65.** Senescence in normal mammalian cell cultures is primarily linked to:
(A) Activation of oncogenes
(B) Overexpression of telomerase
(C) Loss of contact inhibition
(D) Telomere shortening

2017

**66.** During which phase is cell culture most appropriate for subculturing or passaging?
(A) Early lag phase
(B) Late stationary phase
(C) Late log (exponential) phase
(D) Death phase

2024

**67.** Anchorage-dependent cells require what to proliferate?
(A) A liquid suspension environment
(B) A solid, non-toxic substrate for attachment
(C) Continuous magnetic stirring
(D) Complete absence of serum

2016

**68.** Which parameter drastically decreases as a cell culture enters the stationary phase?
(A) Cell volume
(B) Apoptotic rate
(C) Contact inhibition
(D) Mitotic index

2011

**69.** Cellular transformation in vitro generally leads to the loss of:
(A) Contact inhibition
(B) Anchorage independence
(C) Telomerase activity
(D) Nutrient requirements

2019

**70.** Arrange the typical phases of a batch cell culture growth curve:
I. Stationary phase
II. Decline/Death phase
III. Log phase
IV. Lag phase
Choose the correct sequence from the options given below.
(A) IV, III, I, II
(B) III, IV, I, II
(C) IV, I, III, II
(D) I, IV, III, II

2021

**71.** Continuous cell lines are distinct from primary cells because they:
(A) Have bypassed the Hayflick limit and are immortal
(B) Are always derived from embryos
(C) Cannot be frozen for storage
(D) Retain strict contact inhibition

2022

**72.** Which enzyme is typically reactivated or overexpressed in immortalized continuous cell lines?
(A) DNA polymerase alpha
(B) RNA polymerase II
(C) Trypsin
(D) Telomerase

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2013

**73.** Which of the following is a classic characteristic of transformed (tumor) cell lines?
(A) High serum requirement
(B) Anchorage independence
(C) Limited lifespan
(D) Strict monolayer formation

2013

**74.** The HeLa cell line was originally established from which human tissue?
(A) Lung carcinoma
(B) Breast epithelioma
(C) Cervical adenocarcinoma
(D) Renal clear cell carcinoma

2011

**75.** Spontaneous immortalization in vitro occurs most frequently in cells from which species?
(A) Human
(B) Avian
(C) Primate
(D) Mouse

2004

**76.** Viral transformation of cells to create continuous cell lines commonly utilizes:
(A) SV40 (Simian Virus 40)
(B) Influenza virus
(C) Rabies virus
(D) Rotavirus

(C) Chelating agent
(D) Osmotic regulator

2017
**26.** At a low pH (acidic), what color does phenol red become in culture media?
(A) Purple
(B) Red
(C) Yellow
(D) Blue

2022
**27.** HEPES is often added to culture media to:
(A) Provide essential amino acids
(B) Prevent bacterial contamination
(C) Increase medium osmolarity
(D) Buffer pH outside a $CO_2$ incubator

2019
**28.** Fish cell culture media often differs from mammalian media by having:
(A) Lower osmolarity requirements
(B) Higher pH requirements
(C) Higher incubation temperatures
(D) Strictly zero glucose

2021
**29.** Which component provides the main energy source in basal media?
(A) Glutamine
(B) Glucose
(C) Pyruvate
(D) Bicarbonate

2022
**30.** Consider the following statements regarding physicochemical conditions:
I. Mammalian pH is optimally 7.4
II. Fish cells incubate at 37°C
III. Osmolarity is maintained by NaCl
IV. $CO_2$ incubators typically use 5% $CO_2$
V. Phenol red turns fuchsia at pH 6.5
Which of the above statement(s) is/are correct?
(A) I, III and IV
(B) I, II, III and V
(C) II, IV and V
(D) I, IV and V

2021
**31.** Which amino acid is highly unstable in liquid media and often added fresh?
(A) L-Glutamine
(B) L-Arginine
(C) L-Lysine
(D) L-Proline

2006
**32.** DMEM differs from standard MEM primarily by having:
(A) Significantly lower glucose levels
(B) Omission of phenol red indicator
(C) Higher vitamin and amino acid levels
(D) Complete absence of inorganic salts

2024
**33.** Which medium is specifically optimized for growing human lymphocytes?
(A) Ham's $F_{12}$
(B) L-15 (Leibovitz)
(C) Grace's Insect Medium
(D) RPMI-1640

2008
**34.** What is the specific advantage of L-15 (Leibovitz) medium?
(A) It requires a 10% $CO_2$ atmosphere
(B) It contains strictly zero glucose
(C) It grows cells without a $CO_2$ atmosphere
(D) It is an entirely serum-free media

2010
**35.** Natural culture media includes which of the following?
(A) DMEM
(B) Chick embryo extract
(C) RPMI-1640
(D) Ham's $F_{10}$

2001
**36.** Which medium was initially designed for cloning CHO cells at low density?
(A) IMDM
(B) Basal Medium Eagle (BME)
(C) Medium 199
(D) Ham's $F_{12}$

2011
**37.** Which inorganic salt is critical for cellular adhesion in anchorage-dependent cells?
(A) Calcium chloride
(B) Potassium chloride
(C) Sodium phosphate
(D) Magnesium sulfate

2007
**38.** The primary function of sodium bicarbonate in media is to:
(A) Serve as a carbon source
(B) Provide osmotic pressure
(C) Act as a buffering agent with $CO_2$
(D) Prevent microbial growth

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2010

**90.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Organ cultures cannot be expanded by passaging like standard cell cultures.
**Reason (R):** Passaging disrupts the intricate 3D structural organization and intercellular relationships.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2008

**91.** T-flasks used in routine culture are named based on their:
(A) Total volumetric capacity in mL
(B) Temperature requirement
(C) Neck shape
(D) Available surface area for cell attachment in $cm^2$

2022

**92.** A T-75 flask provides approximately what surface area for cell growth?
(A) 7.5 $cm^2$
(B) 75 $cm^2$
(C) 750 $cm^2$
(D) 7500 $cm^2$

2007

**93.** Roller bottle culture systems are primarily designed to:
(A) Induce cellular transformation
(B) Greatly increase available surface area for anchorage-dependent cells
(C) Grow strictly suspension cells
(D) Generate single-cell clones

2017

**94.** What is a key advantage of roller bottles over stationary T-flasks?
(A) Ability to grow cells at 4°C
(B) Better aeration and continuous mixing of media
(C) Complete elimination of serum requirement
(D) They do not require enzymatic dissociation

2012

**95.** Suspension cultures are most appropriate for growing:
(A) Hematopoietic and lymphoid cells
(B) Neuronal cells
(C) Epidermal keratinocytes
(D) Skeletal muscle cells

2004

**96.** Agitation in suspension cultures is crucial to prevent:
(A) Cell division
(B) Viral contamination
(C) Cell settling and clumping
(D) Oxygen over-saturation

2004

**97.** Which material is routinely used to coat plastics to enhance the attachment of fastidious cells?
(A) Trypsin
(B) EDTA
(C) Phenol red
(D) Poly-L-lysine

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2019

**98.** Most modern cell culture flasks are treated with which process to make the surface hydrophilic?
(A) Autoclaving
(B) Siliconization
(C) Tissue-culture (TC) plasma treatment
(D) Gamma irradiation

2018

**99.** The ideal speed for a roller bottle apparatus is typically:
(A) 0.1 - 2 rpm
(B) 50 - 100 rpm
(C) 100 - 200 rpm
(D) 500 - 1000 rpm

2023

**100.** Match the culture vessel in List I with its primary characteristic in List II.
List I: (Vessel)
P. T-flask
Q. Roller bottle
R. Spinner flask
S. Multi-well plate
List II: (Characteristic)
1. Suspension culture via magnetic stirring
2. High surface area via slow rotation
3. High-throughput screening assays
4. Routine stationary monolayer maintenance
(A) P-4, Q-2, R-1, S-3
(B) P-4, Q-1, R-2, S-3
(C) P-2, Q-4, R-1, S-3
(D) P-1, Q-2, R-3, S-4

2016
**77.** Which term describes the occurrence of cells piling up to form a focus in transformed cultures?
(A) Contact inhibition
(B) Loss of contact inhibition
(C) Cellular senescence
(D) Anchorage dependence

2012
**78.** Identify the INCORRECT statement regarding continuous cell lines.
(A) They are easier to maintain than primary cultures
(B) They are highly heterogeneous and exactly mimic in vivo conditions
(C) They often have abnormal karyotypes
(D) They typically grow much faster than primary cells

2023
**79.** CHO cells are a widely used continuous cell line. CHO stands for:
(A) Chinese Hamster Ovary
(B) Canine Heart Outgrowth
(C) Cultured Human Osteoblasts
(D) Chimeric Human Oocytes

2025
**80.** Identify the INCORRECT pair regarding cell lines.
(A) Vero - African green monkey kidney
(B) BHK-21 - Baby hamster kidney
(C) HEK-293 - Human endothelial keratinocytes
(D) Jurkat - Human T lymphocyte

2003
**81.** Organ culture differs from cell culture primarily because it:
(A) Uses single-cell suspensions
(B) Requires much higher trypsin concentrations
(C) Employs completely serum-free media exclusively
(D) Preserves the 3D tissue architecture and cellular interactions

2014
**82.** Which technique is classically used to maintain tissue explants at the liquid-gas interface?
(A) Roller bottles
(B) Micro-carrier culture
(C) Raft or grid method
(D) Hollow-fiber perfusion

2025
**83.** The major limitation of long-term organ culture is:
(A) Rapid cellular transformation
(B) Lack of nutrient and gas diffusion to the center of the tissue
(C) Excessive contact inhibition
(D) Obligatory use of continuous agitation

2017
**84.** In the raft method of organ culture, the tissue is typically supported by:
(A) A magnetic stir bar
(B) Dextran beads
(C) A continuous flow of $CO_2$ gas
(D) A layer of agarose or filter paper

2021
**85.** Organotypic cultures are primarily valuable for studying:
(A) Developmental biology and tissue physiology
(B) High-yield monoclonal antibody production
(C) Large-scale viral vaccine manufacturing
(D) Single-cell karyotyping

2024
**86.** Which feature is generally lost during the transition from organ culture to conventional 2D cell culture?
(A) Cell proliferation
(B) Requirement for basal media
(C) Histological architecture
(D) Cellular senescence

2005
**87.** Embryonic organ culture is highly dependent on precise regulation of:
(A) Morphogen and hormone gradients
(B) Stirring speed
(C) Trypsin concentration
(D) Suspension density

2014
**88.** Why is gas exchange more critical in organ culture than in monolayer culture?
(A) Organ cultures have higher metabolic rates
(B) Organ cultures utilize anaerobic respiration
(C) Monolayer cultures do not require oxygen
(D) Diffusion through 3D tissue mass is highly restricted

2007
**89.** Which system simulates an in vivo-like microenvironment for 3D organ culture?
(A) Stirred-tank bioreactor
(B) Air-liquid interface (ALI) culture
(C) Cytodex microcarrier culture
(D) Cell factory systems

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fibers
(B) Exclusively inside the fiber lumen
(C) Suspended in the main reservoir
(D) Embedded within the plastic casing

2023
**114.** Perfusion culture systems are defined by:
(A) Growth of cells on solid agar
(B) Use of static roller bottles
(C) Continuous supply of medium with simultaneous retention of cells
(D) Complete depletion of nutrients before harvesting

2004
**115.** Hollow-fiber systems uniquely mimic which physiological structure?
(A) Capillary blood vessels
(B) Lymph nodes
(C) Bone marrow
(D) Neural networks

2012
**116.** Cell retention in perfusion bioreactors is commonly achieved using:
(A) High-speed impellers
(B) Trypsinization
(C) Heating elements
(D) Spin filters or acoustic settlers

2022
**117.** What is the standard diameter range for typical microcarrier beads?
(A) 1 - 5 μm
(B) 100 - 300 μm
(C) 1 - 2 mm
(D) 10 - 20 mm

2004
**118.** Which system yields the highest volumetric cell density (cells/mL)?
(A) Standard T-flask
(B) Hollow-fiber perfusion
(C) Stirred tank batch culture
(D) Roller bottles

2021
**119.** A major disadvantage of hollow-fiber systems is:
(A) High shear stress
(B) Low cell densities
(C) Rapid washing away of secreted products
(D) Difficulty in scale-up and monitoring specific cell masses

2005
**120.** Consider the following statements regarding advanced scale-up:
I. Perfusion retains cells while replacing media
II. Hollow-fiber culture provides very low cell densities
III. Microcarriers require gentle agitation
IV. Cytodex is a brand of microcarrier
Which of the above statement(s) is/are INCORRECT?
(A) I and III
(B) II only
(C) III and IV
(D) I, II and IV

2016
**121.** Cell line authentication is critical to rule out:
(A) Cross-contamination with other cell lines (e.g., HeLa)
(B) Mycoplasma contamination
(C) Viral integration
(D) Media expiration

2004
**122.** Which molecular technique is the current gold standard for human cell line authentication?
(A) Western blotting
(B) Isoenzyme analysis
(C) Short Tandem Repeat (STR) profiling
(D) Trypan blue staining

2025
**123.** Karyotyping is primarily used to evaluate a cell line's:
(A) Protein expression
(B) Lipid composition
(C) Mitochondrial function
(D) Chromosomal stability and number

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2011
**124.** Isoenzyme analysis distinguishes cell lines primarily based on:
(A) Tissue type
(B) Passage number
(C) Species of origin
(D) DNA mutations

2012
**125.** The classic morphological evaluation of fibroblasts reveals them to be:
(A) Spherical and non-adherent
(B) Bipolar or multipolar, elongated cells
(C) Cuboidal with tight junctions
(D) Multinucleated giant cells

2005
**126.** G-banding in chromosomal karyotyping typically utilizes which stain?
(A) Eosin

(B) Coomassie Blue
(C) Ethidium Bromide
(D) Giemsa

2008
**127.** Epithelial cells in culture typically display which morphology?
(A) Polygonal patches or sheets
(B) Spindle-shaped bipolar cells
(C) Small, round, suspension cells
(D) Dendritic neural projections

2019
**128.** If a presumed murine cell line exhibits the human LDH isoenzyme band, what is the conclusion?
(A) It is undergoing transformation
(B) It has mutated
(C) It is contaminated with human cells
(D) It is a healthy murine culture

2011
**129.** STR profiling relies on amplifying specific regions of:
(A) Ribosomal RNA
(B) Microsatellite DNA
(C) Mitochondrial DNA
(D) Messenger RNA

2001
**130.** Consider the following statements on cell line authentication:
I. STR profiling is specific to humans and some distinct animal models
II. Isoenzyme analysis cannot differentiate species
III. Karyotyping observes metaphase chromosomes
IV. HeLa contamination is a minor, historical issue
V. Morphological checks are sufficient for formal publication
Which of the above statement(s) is/are correct?
(A) I and III
(B) II, IV and V
(C) I, III and IV
(D) II and V

2022
**131.** The Master Cell Bank (MCB) is derived directly from:
(A) The Working Cell Bank (WCB)
(B) End-oF-production cells
(C) Contaminated backup vials
(D) The initial cloned or established cell population

2017
**132.** What is the fundamental rule regarding the Working Cell Bank (WCB)?
(A) It is generated by expanding one or more vials from the MCB
(B) It must be frozen at -20°C
(C) It is kept entirely at room temperature
(D) It replaces the MCB every year

2022
**133.** Which chemical is the most commonly used cryoprotectant in cell banking?
(A) Ethanol
(B) Dimethyl sulfoxide (DMSO)
(C) Formaldehyde
(D) Polyethylene glycol (PEG)

2017
**134.** DMSO prevents freeze-damage to cells primarily by:
(A) Providing energy during freezing
(B) Preventing intracellular ice crystal formation
(C) Cross-linking membrane proteins
(D) Increasing cell size

2011
**135.** The optimal cooling rate for cryopreserving mammalian cells is generally:
(A) -1°C per minute
(B) -10°C per minute
(C) -50°C per minute
(D) Instant plunging into liquid nitrogen

2009
**136.** Which equipment ensures the correct controlled cooling rate during cryopreservation?
(A) Hemocytometer
(B) Liquid nitrogen dewar
(C) Stirred tank
(D) Mr. Frosty (isopropanol container)

2012
**137.** The standard protocol for reviving frozen cells requires:
(A) Slow thawing at 4°C
(B) Slow thawing at room temperature
(C) Rapid thawing in a 37°C water bath
(D) Rapid thawing in an autoclave

2015
**138.** Why must cells be washed or diluted immediately after thawing?
(A) To remove toxic DMSO
(B) To trigger cell division
(C) To supply high glucose
(D) To sterilize the culture

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2010

**139.** Liquid nitrogen is used for long-term cell storage. What is its temperature?
(A) -80°C
(B) -135°C
(C) -196°C
(D) -273°C

2003

**140.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Cells must be frozen slowly but thawed rapidly.
**Reason (R):** Slow freezing minimizes intracellular ice, and rapid thawing prevents the recrystallization of water that damages membranes.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2014

**141.** The most insidious and difficult-to-detect biological contaminant in cell culture is:
(A) E. coli
(B) Aspergillus niger
(C) Yeast
(D) Mycoplasma

2007

**142.** Mycoplasma contamination is often invisible under standard light microscopy because:
(A) They are perfectly transparent to light
(B) They cause immediate lysis of host cells
(C) They lack cell walls and are very small
(D) They live exclusively within the nucleus

2023

**143.** Which fluorescent dye is frequently used for rapid visual detection of Mycoplasma?
(A) Trypan blue
(B) Hoechst 33258
(C) Propidium iodide
(D) Coomassie blue

2002

**144.** Bacterial contamination in a cell culture typically causes the medium to:
(A) Turn extremely red (alkaline)
(B) Become highly viscous and clear
(C) Evaporate rapidly
(D) Turn yellow (acidic) and become turbid

2023

**145.** Fungal contamination (mold) is easily characterized macroscopically by:
(A) Fuzzy, multicellular mycelial growth floating on the medium
(B) Rapid pH drop
(C) Complete cell lysis within 1 hour
(D) Green fluorescence

2023

**146.** A common source of cross-contamination in laminar flow hoods is:
(A) UV light degradation
(B) Using filtered media
(C) Working with multiple cell lines simultaneously
(D) Pre-warming media

2004

**147.** Which antibiotic combination is historically standard for preventing bacterial contamination in basic cell culture?
(A) Penicillin and Streptomycin
(B) Ampicillin and Kanamycin
(C) Tetracycline and Chloramphenicol
(D) Puromycin and Geneticin

2015

**148.** Why is routine, continuous use of prophylactic antibiotics in cell culture discouraged?
(A) It makes the media too acidic
(B) It digests the cellular matrix
(C) It rapidly degrades phenol red
(D) It masks low-level contamination and promotes resistance

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2003

**149.** Viral contamination of animal cell cultures can sometimes be detected by observing:
(A) Mycelial mats
(B) Cytopathic effects (CPE)
(C) Immediate media turbidity
(D) Plasmolysis

2021

**150.** Match the contaminant in List I with its typical characteristic in culture in List II.
List I: (Contaminant)
P. Bacteria
Q. Yeast
R. Mold
S. Mycoplasma
List II: (Characteristic)
1. Turbid, acidic medium; rods or cocci
2. Ovoid particles; may show budding
3. Fuzzy macroscopic colonies; hyphae

2019

**249.** The isolated variable domain of a heavy-chain-only antibody is commonly referred to as a:
(A) Macrobody
(B) Nanobody
(C) Fab
(D) scFv

2023

**250.** Match the antibody format in List I with its description in List II.
List I: (Format)
P. IgG
Q. Fab
R. scFv
S. Nanobody
List II: (Description)
1. Single VHH domain from camelids
2. Full Y-shaped molecule with 2 Heavy and 2 Light chains
3. VH and VL connected by a peptide linker
4. Result of papain cleavage
(A) P-2, Q-4, R-3, S-1
(B) P-2, Q-3, R-4, S-1
(C) P-1, Q-4, R-3, S-2
(D) P-3, Q-1, R-2, S-4

2001

**251.** Which factor is LEAST likely to affect the success of primary cell isolation?
(A) Age of the source animal
(B) Volume of the $CO_2$ incubator
(C) Type and concentration of dissociating enzyme
(D) Time elapsed between tissue removal and processing

2025

**252.** The specific purpose of adding sodium pyruvate to some basal media is to:
(A) Induce differentiation
(B) Prevent fungal growth
(C) Regulate pH changes
(D) Serve as an intermediate carbon/energy source bypass for glycolysis

2011

**253.** Which serum-derived factor specifically inhibits trypsin activity?
(A) Alpha-1 antitrypsin
(B) Fibronectin
(C) Transferrin
(D) Albumin

2015

**254.** When thawing a cryovial, why is rapid immersion in a 37°C water bath recommended?
(A) To shock the cells into dividing
(B) To instantly vaporize the DMSO
(C) To prevent the formation of damaging secondary ice crystals
(D) To activate serum proteins

2022

**255.** The maximum cell density achievable in a standard T-flask is ultimately limited by:
(A) The available attachment surface area
(B) The available volume of medium
(C) The speed of the incubator fan
(D) The size of the flask neck

2017

**256.** Which cell type is inherently capable of growing in suspension without microcarriers?
(A) Primary human foreskin fibroblasts
(B) CHO-$K_1$ epithelial cells
(C) Endothelial cells
(D) HL-60 leukemia cells

2010

**257.** An "aneuploid" cell line is characterized by:
(A) Exactly 46 chromosomes
(B) An abnormal number of chromosomes
(C) Complete lack of DNA
(D) Inability to divide

2024

**258.** The phenomenon where cells stop proliferating due to high cell density and membrane contact is:
(A) Apoptosis
(B) Necrosis
(C) Contact inhibition
(D) Transformation

2015

**259.** A classic sign of Mycoplasma contamination on a cellular level (e.g., using DAPI stain) is:
(A) Enlarged perfectly spherical nuclei
(B) Immediate rupture of the cell membrane
(C) Formation of syncytia
(D) Extra-nuclear fluorescent particulate matter in the cytoplasm

2014

**260.** Consider the following statements regarding cell viability assays:
I. Trypan blue stains dead cells
II. MTT measures metabolic activity
III. PI stains live cells
IV. LDH release indicates membrane damage

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2007
**163.** After proper centrifugation of blood over Ficoll-Hypaque, the lymphocytes are found:
(A) At the buffy coat interface between the plasma and Ficoll
(B) In the pellet at the very bottom
(C) In the clear upper plasma layer
(D) Mixed thoroughly within the Ficoll layer

2012
**164.** Red blood cells and granulocytes end up where after Ficoll gradient centrifugation?
(A) Suspended in plasma
(B) At the interface
(C) In the pellet at the bottom
(D) Floating at the top

2006
**165.** Substances that induce mitosis and activate lymphocytes in culture are called:
(A) Cytotoxins
(B) Mitogens
(C) Adjuvants
(D) Haptens

2003
**166.** Phytohemagglutinin (PHA) primarily stimulates which cell population?
(A) B lymphocytes
(B) Macrophages
(C) Erythrocytes
(D) T lymphocytes

2024
**167.** Which of the following is a classic B-cell mitogen in mice?
(A) Lipopolysaccharide (LPS)
(B) Concanavalin A (ConA)
(C) Phytohemagglutinin (PHA)
(D) Pokeweed mitogen (PWM)

2016
**168.** Lymphocyte proliferation in response to mitogens is classically quantified by incorporating:
(A) Trypan blue
(B) Ethidium bromide
(C) Tritiated thymidine ([3H]-TdR)
(D) Crystal violet

2003
**169.** Concanavalin A (ConA) is derived from:
(A) Jack bean
(B) Red kidney bean
(C) E. coli
(D) Pokeweed

2015
**170.** Arrange the steps of peripheral blood mononuclear cell (PBMC) isolation in the correct sequence:
I. Centrifuge without brake
II. Layer diluted blood carefully over Ficoll
III. Dilute whole blood with PBS
IV. Collect the cloudy interface layer
Choose the correct sequence from the options given below.
(A) III, II, I, IV
(B) II, III, I, IV
(C) III, I, II, IV
(D) II, I, III, IV

2023
**171.** Cellular micromanipulation to introduce DNA into a specific cell nucleus is called:
(A) Electroporation
(B) Transfection
(C) Fusion
(D) Microinjection

2018
**172.** Polyethylene glycol (PEG) promotes cell fusion by:
(A) Altering nuclear DNA
(B) Dehydrating the cell surfaces and disrupting lipid bilayers
(C) Supplying excessive ATP
(D) Dissolving the cytoskeleton

2011
**173.** Which virus is classically used to induce fusion between mammalian cells?
(A) Adenovirus
(B) SV40
(C) Sendai virus
(D) Epstein-Barr virus

2017
**174.** Electrofusion utilizes what mechanism to fuse cells?
(A) Short, high-intensity electric pulses to cause membrane breakdown
(B) High temperature spikes
(C) Continuous low-voltage currents
(D) Magnetic fields

2005
**175.** The resulting fused cell containing two distinct nuclei before they merge is called a:
(A) Hybridoma
(B) Cybrid
(C) Syncytium
(D) Heterokaryon

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4. Invisible by light microscopy; alters metabolism

(A) P-1, Q-2, R-3, S-4
(B) P-2, Q-1, R-4, S-3
(C) P-3, Q-4, R-1, S-2
(D) P-1, Q-3, R-2, S-4

2016

**151.** The principle of the Trypan blue dye exclusion test is based on the fact that:
(A) Live cells actively transport the dye inside
(B) The dye specifically binds to DNA in live cells
(C) The dye reacts with cellular ATP
(D) Dead cells with damaged membranes take up the dye

2021

**152.** When viewing a Trypan blue-treated sample under a microscope, dead cells appear:
(A) Clear and shiny
(B) Red
(C) Blue
(D) Green

2002

**153.** The MTT assay is used primarily to measure:
(A) DNA fragmentation
(B) Cellular metabolic activity / Viability
(C) Cell membrane integrity
(D) Specific protein secretion

2015

**154.** In the MTT assay, viable cells convert the soluble yellow MTT tetrazolium salt into:
(A) Soluble blue resazurin
(B) Red phenol indicator
(C) Green fluorescent GFP
(D) Insoluble purple formazan crystals

2001

**155.** Which enzyme is responsible for the conversion of MTT to its visible product?
(A) Mitochondrial succinate dehydrogenase
(B) Membrane ATPase
(C) Cytosolic hexokinase
(D) Nuclear RNA polymerase

2015

**156.** The XTT assay offers which distinct advantage over the classic MTT assay?
(A) It requires no incubation time
(B) It specifically counts dead cells only
(C) The formazan product is water-soluble, skipping the solubilization step
(D) It uses radioactive isotopes for higher sensitivity

2002

**157.** The release of Lactate Dehydrogenase (LDH) into the culture medium is a classic marker for:
(A) Loss of membrane integrity and cytotoxicity
(B) Exponential cell division
(C) Increased ATP synthesis
(D) Successful transformation

2009

**158.** In fluorescence-based viability assays, Propidium Iodide (PI) specifically stains:
(A) Live cells only
(B) Only apoptotic cells
(C) Mitochondria
(D) Dead cells with compromised membranes

2017

**159.** Calcein-AM is used in viability assays because it fluoresces green only when cleaved by:
(A) Trypsin
(B) Intracellular esterases in live cells
(C) Extracellular lipases
(D) Caspases in dead cells

2001

**160.** Select the correct sequence of steps for conducting an MTT assay.
(A) Seed cells → Add drug → Incubate → Add MTT → Solubilize → Read Absorbance
(B) Add MTT → Seed cells → Solubilize → Add drug → Read Absorbance
(C) Solubilize → Seed cells → Add MTT → Add drug → Read Absorbance
(D) Seed cells → Add MTT → Solubilize → Add drug → Read Absorbance

2023

**161.** Density gradient centrifugation using Ficoll-Hypaque is widely used to isolate:
(A) Adherent fibroblasts
(B) Red blood cells
(C) Intact hepatocytes
(D) Mononuclear cells (lymphocytes and monocytes) from whole blood

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2008

**162.** The specific gravity of standard Ficoll-Hypaque used for human PBMC isolation is:
(A) 1.000 g/mL
(B) 1.077 g/mL
(C) 1.150 g/mL
(D) 1.200 g/mL

(D) Myeloma-myeloma homokaryons

2017

**189.** If a mutant cell line is resistant to the toxic analogue 8-azaguanine, it is usually:
(A) TK-
(B) DHFR-
(C) Aminopterin resistant
(D) HGPRT-

2016

**190.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** HGPRT-deficient cells survive in normal media but die in HAT media.
**Reason (R):** In normal media they use the de novo pathway; in HAT media, aminopterin blocks the de novo pathway and they lack the salvage enzyme.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2014

**191.** Hybridoma technology specifically produces which type of antibodies?
(A) Polyclonal antibodies
(B) Monoclonal antibodies
(C) Autoantibodies
(D) Chimeric antibodies only

2023

**192.** The animal most frequently immunized to provide spleen cells for hybridoma generation is the:
(A) Mouse (typically BALB/c)
(B) Rabbit
(C) Goat
(D) Horse

2016

**193.** Sp2/0 and NS-1 are common examples of what in hybridoma technology?
(A) Adjuvants
(B) Fusion promoters
(C) HGPRT-deficient myeloma cell lines
(D) Reporter genes

2002

**194.** A crucial feature of an ideal myeloma partner cell line is that it should:
(A) Secrete its own heavy and light chains
(B) Be a primary cell
(C) Have a strict anchorage dependence
(D) Be non-secretory for endogenous antibodies

2008

**195.** What is the typical recommended ratio of spleen cells to myeloma cells for PEG fusion?
(A) 1:100 to 1:10
(B) 1:1
(C) 5:1 to 10:1
(D) 100:1 to 500:1

2010

**196.** The purpose of administering a "booster" immunization 3 days before cell fusion is to:
(A) Eradicate all regulatory T cell populations
(B) Induce systemic immunological tolerance
(C) Stimulate antigen-specific B cell proliferation
(D) Eradicate the pool of memory B cells

2022

**197.** Feeder cells (like murine peritoneal macrophages) are often added during initial hybridoma plating to:
(A) Supply growth factors and improve survival at low cell densities
(B) Provide HGPRT to the myelomas
(C) Consume aminopterin
(D) Phagocytose the fused cells

2011

**198.** Which statement about polyclonal antibodies is correct when compared to monoclonals?
(A) They recognize only a single epitope
(B) They are secreted by a single B-cell clone
(C) They offer eternal batch-to-batch consistency
(D) They are derived from multiple B-cell clones recognizing different epitopes

2006

**199.** What role does an adjuvant (e.g., Freund's Complete Adjuvant) play in the immunization protocol?
(A) It fuses the cells
(B) It drastically enhances the immune response to the injected antigen
(C) It specifically targets the bone marrow
(D) It neutralizes HAT medium

2014

**200.** Match the hybridoma component in List I with its primary characteristic in List II.
List I: (Component)
P. Spleen B-cell
Q. Myeloma cell
R. PEG

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S. Aminopterin

List II: (Characteristic)

1. Provides immortality
2. Blocks de novo nucleotide synthesis
3. Provides specific antibody secretion
4. Induces membrane fusion

(A) P-3, Q-1, R-4, S-2
(B) P-1, Q-3, R-4, S-2
(C) P-3, Q-4, R-1, S-2
(D) P-1, Q-2, R-4, S-3

2010

**201.** The process of isolating a single hybridoma cell to establish a uniform, monoclonal population is called:
(A) Transformation
(B) Cryopreservation
(C) Immortalization
(D) Sub-cloning

2010

**202.** Which technique is most universally used for sub-cloning hybridomas?
(A) Limiting dilution
(B) Gradient centrifugation
(C) Western blotting
(D) Electroporation

2012

**203.** In the limiting dilution technique, cells are diluted to a statistical target of:
(A) 0.5 to 1 cell per well
(B) 10 cells per well
(C) 100 cells per well
(D) 1000 cells per well

2002

**204.** Why is sub-cloning performed as early as possible after hybridoma identification?
(A) To prevent loss of essential CD4 receptors
(B) To increase total culture volume significantly
(C) To effectively clear remaining HAT medium
(D) To ensure clonality and prevent overgrowth

2023

**205.** Soft agar cloning relies on which cellular property of hybridomas?
(A) Anchorage independence
(B) Anchorage dependence
(C) Contact inhibition
(D) Density-dependent growth

2014

**206.** Which method is standard for high-throughput screening of hybridoma supernatants for antibody secretion?
(A) Polymerase Chain Reaction (PCR)
(B) Enzyme-Linked Immunosorbent Assay (ELISA)
(C) Mass spectrometry
(D) Karyotyping

2006

**207.** In an indirect ELISA used for screening, the microtiter plate is initially coated with:
(A) The specific antigen of interest
(B) The hybridoma supernatant
(C) An enzyme-linked secondary antibody
(D) A colorimetric substrate

2003

**208.** Flow cytometry can be used to screen hybridomas if the target antigen is:
(A) A soluble secreted protein
(B) A small hapten
(C) A cell-surface associated molecule
(D) An RNA fragment

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2018

**209.** During screening, what indicates a positive well in a standard colorimetric ELISA?
(A) Development of a visible color change upon adding substrate
(B) Formation of a cell pellet
(C) Cell death in the well
(D) Emission of fluorescence

2024

**210.** Consider the following statements regarding sub-cloning:

I. Ensures monoclonality
II. Limiting dilution aims for $> 5$ cells/well
III. Can be performed using flow cytometry sorting (FACS)

Which of the above statement(s) is/are correct?
(A) I and II
(B) I and III
(C) II and III
(D) I only

2003

**211.** Determining the specific class and subclass of a monoclonal antibody is known as:
(A) Epitope mapping
(B) Karyotyping
(C) Humanization
(D) Isotyping

2025

**212.** Most murine monoclonal antibodies generated are of which isotype?
(A) IgA

(B) IgE
(C) IgG
(D) IgM

2008

**213.** Which bacterial protein is widely used for the affinity purification of IgG monoclonal antibodies?
(A) Protein A or Protein G
(B) Trypsin
(C) Streptavidin
(D) Collagenase

2024

**214.** Epitope mapping determines:
(A) The exact DNA sequence of the antibody
(B) The location of the antibody gene on the chromosome
(C) The lifespan of the hybridoma
(D) The precise binding site of the antibody on the target antigen

2015

**215.** Monoclonal antibodies can be cleaved into Fab and Fc fragments by which enzyme?
(A) Papain
(B) Pepsin
(C) Trypsin
(D) Ligase

2023

**216.** Pepsin digestion of an IgG molecule primarily yields:
(A) Two identical Fab fragments
(B) Intact heavy chains only
(C) One F(ab')2 fragment
(D) Free light chains

2009

**217.** The primary advantage of using a Fab fragment over a whole antibody in certain assays is:
(A) Improved tissue penetration due to smaller size
(B) Increased complement activation
(C) Longer halF-life in serum
(D) Bivalent binding

2001

**218.** Affinity chromatography for purifying monoclonal antibodies typically elutes the bound antibody using:
(A) High temperature
(B) Pure distilled water
(C) Organic solvents
(D) A low pH buffer (e.g., pH 2.5 - 3.0)

2014

**219.** Isotyping is rapidly performed using which specific technique?
(A) Density gradient centrifugation
(B) Lateral flow immunoassay or ELISA
(C) MTT assay
(D) Southern blotting

2007

**220.** Consider the following statements about MAb characterization:
I. Papain yields two Fab fragments and one Fc fragment
II. Protein G binds strongly to the Fc region of IgG
III. Epitope mapping alters the antibody structure
IV. IgM is the most common isotype for mature monoclonals
Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) III and IV
(D) I and IV

2009

**221.** The first FDA-approved therapeutic monoclonal antibody, $OKT_3$, targeted which receptor to prevent transplant rejection?
(A) CD20
(B) CD3
(C) HER2
(D) VEGF

2003

**222.** Rituximab is widely used in cancer immunotherapy. It targets which antigen on B-cells?
(A) CD20
(B) CD4
(C) CD8
(D) CD56

2002

**223.** Monoclonal antibodies conjugated to a radioactive isotope or cytotoxic drug are called:
(A) Naked antibodies
(B) Chimeric antibodies
(C) Antibody-drug conjugates (ADCs)
(D) Bispecific antibodies

2007

**224.** Trastuzumab (Herceptin) is an engineered monoclonal antibody used specifically to treat:
(A) Rheumatoid arthritis
(B) Asthma

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(C) Multiple sclerosis
(D) HER2-positive breast cancer

2001
**225.** In over-the-counter home pregnancy tests, monoclonal antibodies are used to detect:
(A) Insulin
(B) Estrogen
(C) Human chorionic gonadotropin (hCG)
(D) Testosterone

2009
**226.** Which application is an example of an in vitro diagnostic use of MAbs?
(A) Targeted tumor cell killing
(B) Blood group typing (ABO)
(C) Neutralizing snake venom in a patient
(D) Modulating autoimmune responses

2025
**227.** Checkpoint inhibitors, a breakthrough in cancer therapy, utilize MAbs that typically target:
(A) CD20 or CD19
(B) EGF or VEGF
(C) Insulin receptors
(D) PD-1, PD-$L_1$, or CTLA-4

2013
**228.** Anti-TNF-alpha monoclonal antibodies (e.g., Infliximab) are primarily used to treat:
(A) Autoimmune and inflammatory diseases
(B) Viral infections
(C) Hemophilia
(D) Bacterial sepsis

2002
**229.** A significant drawback of using pure murine (mouse) monoclonal antibodies therapeutically in humans is:
(A) They possess an insufficiently small size
(B) They cannot bind to human target antigens
(C) They trigger a systemic HAMA response
(D) They do not cross the blood-brain barrier

2024
**230.** Consider the following pairs of MAbs and targets:
I. Rituximab - CD20
II. Trastuzumab - HER2
III. Bevacizumab - VEGF
IV. Infliximab - CD3
V. Pembrolizumab - PD-1
Which of the above pair(s) is/are INCORRECT?
(A) IV only
(B) I and V
(C) II and III
(D) IV and V

2013
**231.** To reduce the immunogenicity of murine antibodies, scientists developed chimeric antibodies which consist of:
(A) Murine constant regions and human variable regions
(B) Human constant regions and murine variable regions
(C) Only human variable regions
(D) Only murine constant regions

2003
**232.** In standard nomenclature, a chimeric monoclonal antibody typically ends with the suffix:
(A) -ximab
(B) -omab
(C) -zumab
(D) -umab

2004
**233.** Humanized monoclonal antibodies are engineered so that:
(A) Only the constant regions are murine
(B) The entire sequence is 100% human
(C) Only the Complementarity-Determining Regions (CDRs) are murine
(D) They are produced in human bone marrow

2025
**234.** The process of transferring murine CDRs onto a human antibody framework is called:
(A) Isotype switching
(B) Somatic hypermutation
(C) Phage display
(D) CDR grafting

2010
**235.** A fully human monoclonal antibody (suffix -umab) can be generated by:
(A) Using transgenic mice possessing human immunoglobulin genes
(B) Immunizing normal BALB/c mice
(C) Extracting hybridomas from humans
(D) Standard PEG fusion of mouse spleen and myeloma cells

2007
**236.** What percentage of a humanized antibody (e.g., -zumab) is typically of human origin?
(A) 50%
(B) 65%
(C) ~90-95%
(D) 100%

2025
**237.** Which strategy is used to completely avoid animals in the generation of human antibodies?

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(A) Ascites production
(B) In vitro phage display library screening
(C) Humanizing mouse hybridomas
(D) CDR grafting

2009
**238.** Bispecific antibodies are uniquely engineered to:
(A) Evade the immune system completely
(B) Survive boiling temperatures
(C) Lack a heavy chain entirely
(D) Bind to two completely different antigens or epitopes simultaneously

2008
**239.** A "designer antibody" built to bring a cytotoxic T-cell into direct contact with a tumor cell is typically a:
(A) Bispecific T-cell engager (BiTE)
(B) Chimeric antibody
(C) Fab fragment
(D) Polyclonal serum

2005
**240.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Chimeric antibodies are less likely to induce a HAMA response than pure murine antibodies.
**Reason (R):** Chimeric antibodies have human constant regions, which constitute the majority of the antibody's highly immunogenic structure.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2003
**241.** A single-chain variable fragment (scFv) is composed of:
(A) One entire heavy chain including constant regions
(B) The isolated CDR loops of the antibody
(C) Two identical Fab fragments joined together
(D) VH and VL regions joined by a peptide linker

2014
**242.** What is a primary advantage of scFv molecules over full-sized monoclonal antibodies?
(A) They activate complement more strongly
(B) They have much longer serum halF-lives
(C) They are small enough to easily penetrate dense solid tumors
(D) They possess two binding sites

2022
**243.** In phage display technology, the gene encoding the antibody fragment is inserted into the phage genome so that the antibody fragment is:
(A) Secreted into the media
(B) Expressed on the surface coat of the bacteriophage
(C) Stored inside the phage capsid
(D) Destroyed by the bacteria

2021
**244.** The process of isolating phages through repeated cycles of binding, washing, and amplification is called:
(A) Grafting of murine CDRs to human framework
(B) Blotting of proteins to nitrocellulose membrane
(C) Splicing of antibody genes into the genome
(D) Iterative biopanning of phage display libraries

2010
**245.** Which microorganism is typically used to host the bacteriophages in phage display libraries?
(A) Escherichia coli
(B) Saccharomyces cerevisiae
(C) Bacillus subtilis
(D) Pichia pastoris

2010
**246.** Recombinant antibodies can be rapidly mass-produced in completely serum-free systems using:
(A) Mice ascites
(B) Human volunteers
(C) Transfected CHO cells or E. coli
(D) Hybridomas only

2016
**247.** A "diabody" is a recombinant antibody format consisting of:
(A) Two scFvs linked together
(B) A full IgG with no light chains
(C) A single heavy chain domain
(D) An ADC conjugate

2011
**248.** Camels and llamas produce a unique class of functional antibodies that naturally lack:
(A) Heavy chains
(B) Variable regions
(C) Disulfide bonds
(D) Light chains

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2019
**176.** What is the primary advantage of electrofusion over PEG fusion?
(A) It requires no specialized equipment
(B) It offers higher fusion efficiency and lower cellular toxicity
(C) It has higher toxicity
(D) It works without cells touching

2001
**177.** A cybrid is a unique fusion product consisting of:
(A) Two intact whole cells
(B) Two nuclei without cytoplasm
(C) A whole cell fused with an enucleated cytoplast
(D) A cell and a virus

2014
**178.** Dielectrophoresis in electrofusion is used specifically to:
(A) Align the cells into "pearl chains" prior to the fusion pulse
(B) Lyse the cell membrane
(C) Extract the nucleus
(D) Stain the cells

2019
**179.** Sendai virus induces cell fusion via specific viral envelope proteins. The virus is usually prepared by:
(A) Boiling to denature its DNA
(B) Freezing at -20°C
(C) Treating with strong acids
(D) UV irradiation to inactivate its replication while preserving fusion capability

2002
**180.** Which of the following is a disadvantage of using Polyethylene Glycol (PEG) for cell fusion?
(A) It requires extremely expensive specialized equipment
(B) It is highly toxic to cells upon prolonged exposure
(C) It only works on plant protoplasts, not animal cells
(D) It requires the use of live, replicating viruses

2017
**181.** In somatic cell genetics, the de novo synthesis pathway of nucleotides can be blocked by:
(A) Hypoxanthine
(B) Aminopterin
(C) Thymidine
(D) Glutamine

2002
**182.** Which enzyme represents the purine salvage pathway required to survive aminopterin blockage?
(A) Thymidine kinase (TK)
(B) Dihydrofolate reductase (DHFR)
(C) DNA polymerase
(D) Hypoxanthine-guanine phosphoribosyltransferase (HGPRT)

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2011
**183.** Cells utilize the salvage pathway only when:
(A) Hypoxanthine and Thymidine are provided in the medium
(B) The de novo pathway is active
(C) Aminopterin is absent
(D) They are dividing exponentially

2005
**184.** In HAT selection, what does the "H" stand for?
(A) Histidine
(B) Heparin
(C) Hypoxanthine
(D) Hyaluronidase

2008
**185.** A cell line that is TK-deficient (TK-) cannot salvage which precursor?
(A) Thymidine
(B) Hypoxanthine
(C) Adenine
(D) Guanine

2015
**186.** Why do unfused myeloma cells die in HAT medium during hybridoma generation?
(A) They are normal cells undergoing senescence
(B) They are killed by the PEG used for fusion
(C) They are unable to absorb external thymidine
(D) They lack the HGPRT enzyme for salvage

2010
**187.** Why do unfused splenic B-cells eventually die in HAT medium?
(A) They are HGPRT deficient
(B) They are primary cells with a limited lifespan and die naturally
(C) Aminopterin is directly toxic to their membranes
(D) They lack thymidine kinase

2023
**188.** Which cells survive HAT selection in a standard hybridoma fusion?
(A) Unfused myeloma cells
(B) Unfused spleen cells
(C) Spleen-myeloma hybrid cells (hybridomas)

V. Calcein-AM stains dead cells

Which of the above statement(s) is/are correct?

(A) I, II and IV
(B) I, III and V
(C) II, III and IV
(D) I, II and V

2008

**261.** Cell synchronization can be achieved by removing which component from the culture medium?

(A) Glucose
(B) Serum
(C) Amino acids
(D) Phenol red

2001

**262.** Which method chemically induces cell synchronization by reversibly arresting cells in the S phase?

(A) Serum starvation
(B) Trypsinization
(C) PEG treatment
(D) Double thymidine block

2024

**263.** A laminar flow hood provides a sterile environment primarily through:

(A) High-efficiency particulate air (HEPA) filtration of the air supply
(B) Continuous UV irradiation during work
(C) Ozone generation
(D) High-temperature air currents

2013

**264.** What is the standard classification of a biological safety cabinet suitable for routine, non-hazardous animal cell culture?

(A) Class I
(B) Class III
(C) Class II
(D) Class IV

2010

**265.** In a Class II Type $A_2$ biological safety cabinet, what percentage of the air is typically recirculated?

(A) 0%
(B) 30%
(C) 70%
(D) 100%

2018

**266.** A Class III biological safety cabinet is often referred to as a:

(A) Fume hood
(B) Clean bench
(C) Laminar flow hood
(D) Glove box

2013

**267.** The "passage number" of a cell line refers to:

(A) The number of times the cells have been thawed
(B) The number of times the culture has been subcultured (split)
(C) The number of days the cells have been in culture
(D) The specific incubator it is housed in

2013

**268.** High passage numbers in continuous cell lines are associated with:

(A) Increased genetic stability
(B) Return to primary cell characteristics
(C) Phenotypic and genetic drift
(D) Enhanced contact inhibition

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2021

**269.** A hemocytometer is specifically used for:

(A) Measuring pH
(B) Determining osmolarity
(C) Assessing protein concentration
(D) Quantifying cell concentration (cells/mL)

2013

**270.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).

**Assertion (A):** It is recommended to perform Mycoplasma testing routinely.

**Reason (R):** Mycoplasma grows slowly, causes no visible turbidity, but profoundly alters cell metabolism and gene expression.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2018

**271.** When counting cells using a standard hemocytometer, the volume of one large corner square is:

(A) 0.0001 mL ($10^{-4}$ mL)
(B) 0.1 mL
(C) 1 mL
(D) 10 µL

2008

**272.** If you count an average of 50 cells in a standard large square, what is the concentration?

enzymes involved in DNA synthesis and energy metabolism. Transferrin binds to ferric ions in the medium and delivers them via receptor-mediated endocytosis. This ensures that cells have a steady supply of iron, which is vital for health.

**49.** (D) Easier downstream purification of products
A direct advantage of serum-free media is the easier downstream purification of products. Since these media lack the high concentrations of varied proteins found in serum, the desired product, such as a recombinant protein or monoclonal antibody, can be isolated more efficiently. This simplification reduces processing time and costs while increasing the purity and consistency of the final biological therapeutic product.

**50.** (A) P-3, Q-1, R-2, S-4
Different culture supplements serve specific physiological roles: insulin stimulates glucose uptake and metabolism, while fibronectin is critical for cell attachment to the substrate. Ethanolamine is essential for the synthesis of phospholipids in the cell membrane, and selenium acts as a necessary cofactor for antioxidant enzymes like glutathione peroxidase. Correctly matching these supplements to their functions is vital for optimization.

**51.** (B) Primary culture
The process of isolating cells directly from tissue to establish a culture is called primary culture. These cells are taken from a living organism and maintained in an artificial environment. Primary cultures initially reflect the characteristics of the source tissue but have a limited lifespan. They serve as the starting point for developing cell lines through subsequent subculturing and expansion.

**52.** (D) Trypsin
Trypsin is the most widely used enzyme for the dissociation of tissues into single cells. It is a serine protease that cleaves proteins at specific sites, effectively breaking the junctions that hold cells together and to the extracellular matrix. By treating minced tissue with a dilute trypsin solution, researchers can obtain a suspension of viable individual cells ready for laboratory culture.

**53.** (A) Soft tissues like spleen or brain
Mechanical disruption is most suitable for soft tissues like the spleen or brain, which have minimal connective tissue. This method involves physically mincing or sieving the tissue to release cells into a suspension. While faster than enzymatic digestion, it can be harsher on cells. However, for fragile tissues with loose structures, it provides a simple way to obtain cells.

**54.** (C) Fibrous and connective tissues
Collagenase is preferred over trypsin for dissociating fibrous and connective tissues. Unlike trypsin, which is a general protease, collagenase specifically targets collagen, the primary structural protein in the extracellular matrix. This specificity allows for the effective release of cells from dense tissues like skin, heart, or lungs while causing less damage to sensitive cell surface proteins, resulting in higher viability.

**55.** (A) To chelate calcium and magnesium, weakening cell junctions
EDTA is added during enzymatic dissociation to chelate divalent cations like calcium and magnesium. These ions are essential for the stability of cell-cell adhesion molecules, such as cadherins. By removing these ions, EDTA weakens the junctions between cells, making it easier for enzymes like trypsin to access and break down the protein bridges, thereby facilitating the creation of a suspension.

**56.** (D) Outgrowth and migration from the tissue
The explant culture method relies on the natural ability of cells to migrate and grow out from a small piece of tissue placed on a surface. As the tissue adheres to the culture vessel, cells at the edges begin to crawl onto the substrate, eventually forming a monolayer. This technique preserves some tissue architecture and is useful when enzymatic dissociation is undesirable.

**57.** (B) Warm enzymatic dissociation
Warm enzymatic dissociation yields the highest initial number of viable cells from a tissue sample. By incubating minced tissue with enzymes like trypsin or collagenase at 37 degrees Celsius, the extracellular matrix is rapidly broken down. This efficient process releases a large population of individual cells. While the heat increases enzymatic activity, care must be taken to prevent over-digestion.

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2011
**285.** A disadvantage of using excessively high concentrations of trypsin is:
(A) Proteolytic degradation of essential cell surface receptors
(B) Slower cell detachment
(C) Inactivation of the buffering system
(D) Premature differentiation

2011
**286.** What represents a "confluent" monolayer in cell culture?
(A) Cells floating freely in the medium
(B) Cells strictly in the lag phase
(C) Cells completely covering the available surface area with no empty spaces
(D) The exact moment cells double in number

2009
**287.** Contact inhibition is primarily mediated by which type of cell-cell junctional proteins?
(A) Integrins
(B) Selectins
(C) Connexins
(D) Cadherins

2003
**288.** To preserve maximum viability, a culture should ideally be subcultured when it reaches what confluency?
(A) 10 - 20%
(B) 40 - 50%
(C) 70 - 85%
(D) 100% and piling up

2021
**289.** The term "epithelioid" describes cultured cells that:
(A) Look like long, thin spindles
(B) Are multinucleated
(C) Resemble epithelial cells (cuboidal/ polygonal) but their exact origin is unconfirmed
(D) Grow exclusively in suspension

2021
**290.** Consider the following cell morphologies:
I. Fibroblastic: bipolar, elongated
II. Epithelial-like: polygonal, distinct patches
III. Lymphoblast-like: spherical, suspension
Which of the above matches are correct?
(A) I and II
(B) I and III
(C) II and III
(D) I, II and III

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2012
**291.** Which of the following is a classic indicator of early apoptosis in cultured cells?
(A) Membrane blebbing
(B) Complete cellular lysis
(C) Massive swelling
(D) Rapid proliferation

2010
**292.** Necrosis in cell culture is typically characterized by:
(A) Orderly DNA fragmentation
(B) Shrinkage and formation of apoptotic bodies
(C) Cellular swelling and random membrane rupture
(D) Increased ATP production

2023
**293.** The Ames test on cultured cells evaluates:
(A) Cell cycle speed
(B) Mutagenic potential of a compound
(C) Hybridoma fusion efficiency
(D) Viral load

2013
**294.** In the context of large-scale production, "downstream processing" refers to:
(A) The initial thawing of the cells
(B) The fermentation process itself
(C) Sterilization of the bioreactor
(D) The extraction and purification of the desired product (e.g., antibody)

2002
**295.** Which type of chromatography is generally the first step in downstream processing of MAbs?
(A) Protein A affinity chromatography
(B) Size exclusion chromatography
(C) Ion exchange chromatography
(D) Hydrophobic interaction chromatography

2004
**296.** A major ethical and scientific push in modern cell culture is the replacement of FBS with:
(A) Human serum
(B) Chick embryo extract
(C) Chemically defined, animal-component-free media
(D) Unfiltered plasma

2017
**297.** The term "in vitro" literally translates to:
(A) In glass
(B) In life
(C) In plastic
(D) In tissue

2001

**298.** Which component is strictly essential for in vitro translation systems derived from cells?
(A) Intact mitochondria
(B) Cell wall
(C) Lysosomes
(D) Ribosomes

2022

**299.** High-throughput screening (HTS) using cultured cells relies heavily on:
(A) Roller bottles
(B) Large stirred-tank bioreactors
(C) Whole animal models
(D) 96, 384, or 1536-well microtiter plates

2022

**300.** The ultimate goal of optimizing a fed-batch bioreactor for hybridomas is to maximize:
(A) The volumetric titer (mg/L) of the secreted monoclonal antibody
(B) The speed of the lag phase
(C) The size of the individual cells
(D) The rate of cellular senescence

**********

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# ANSWERS

**1.** (D) Ross Harrison
Ross Harrison pioneered the first successful animal cell culture using frog nerve fibers in 1907. This experiment demonstrated that nerve fibers could grow outside the body in a clotted lymph medium, proving that they originate from single cells. This foundational work paved the way for modern cell biology and tissue culture techniques used in laboratories today.

**2.** (C) Establishment of the HeLa cell line
George Gey achieved a major milestone in 1951 by establishing the HeLa cell line. This was the first human immortalized cell line, derived from cervical cancer cells of Henrietta Lacks. These cells exhibit robust growth and have been instrumental in vaccine development, cancer research, and numerous medical breakthroughs, maintaining their viability indefinitely through continuous laboratory subculturing.

**3.** (B) Kohler and Milstein
Kohler and Milstein developed hybridoma technology in 1975 to produce monoclonal antibodies. This technique involves fusing short-lived, antibody-producing B cells with immortal myeloma cells. The resulting hybrid cells inherit both specific antibody production and the ability to grow indefinitely in culture. Their work revolutionized diagnostics and therapeutics, earning them the Nobel Prize in Physiology and Medicine.

**4.** (D) He invented the first chemically defined medium
Alexis Carrel made significant contributions to cell culture but did not invent the first chemically defined medium. He is primarily known for developing aseptic techniques and the flask culture method. His long-term maintenance of chick heart tissue demonstrated that cells could survive for decades. Chemically defined media were actually developed later by other researchers such as Harry Eagle.

**5.** (A) Basal growth media
Harry Eagle is recognized for developing basal growth media, specifically Minimum Essential Medium. He identified the precise nutrients, including specific amino acids, vitamins, and salts, required for animal cells to proliferate in vitro. His systematic studies simplified culture requirements, allowing researchers to support diverse cell types using standardized, reproducible formulations that serve as the foundation for modern media.

**6.** (C) Chemically defined growth factors
The discovery of chemically defined growth factors and hormones allowed researchers to eliminate the need for feeder layers in many cell cultures. Feeder layers were originally used to provide essential nutrients and signals. By identifying and adding these specific components directly to the media, scientists achieved more controlled environments, reducing variability and contamination risks in various cellular experiments.

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**7.** (A) Aseptic cell manipulation
Laminar flow biological safety cabinets drastically improved aseptic cell manipulation by providing a controlled, sterile workspace. These cabinets use HEPA filters to create a uniform flow of clean air, preventing contaminants from entering the work area while protecting the operator. This advancement significantly reduced the risk of microbial contamination, ensuring higher success rates and reproducibility in cultures.

**8.** (D) Tissue plasminogen activator
Tissue plasminogen activator was the first commercially produced recombinant protein derived from mammalian cells. Approved in the 1980s, it is used to treat heart attacks and strokes by dissolving blood clots. Mammalian cells, specifically Chinese Hamster Ovary cells, were utilized because they provide the complex post-translational modifications, like glycosylation, necessary for the protein to function effectively.

**9.** (B) Enders - Polio virus in culture
John Enders is credited with the milestone of successfully growing the polio virus in non-neural cell cultures. This achievement was critical for the subsequent development of the polio vaccine by Salk and Sabin. His work demonstrated that viruses could be propagated in laboratory-grown

the citric acid cycle to produce ATP. This energy is essential for all cellular processes, including growth, division, and protein synthesis. Most standard media formulations include glucose to support the high metabolic demands of cells.

**30.** (A) I, III and IV
Mammalian cells grow best at pH 7.4, and osmolarity is primarily maintained by salts like sodium chloride. $CO_2$ incubators typically use a 5% concentration to stabilize bicarbonate buffers. Fish cells require lower temperatures, not 37 degrees. Phenol red turns yellow at acidic pH levels, not fuchsia. Therefore, statements I, III, and IV accurately describe standard culture conditions.

**31.** (A) L-Glutamine
L-Glutamine is highly unstable in liquid media because it spontaneously degrades into ammonia and pyrrolidone carboxylic acid. Ammonia is toxic to cells, and the loss of glutamine deprives them of an essential nitrogen and energy source. Consequently, glutamine is often added fresh to the medium just before use to ensure that the cells have adequate nutrients for healthy proliferation.

**32.** (C) Higher vitamin and amino acid levels
Dulbecco's Modified Eagle Medium differs from standard Minimum Essential Medium by containing significantly higher concentrations of vitamins and amino acids. It was developed to support a wider range of cell types, including high-density cultures. Additionally, it often contains four times the glucose concentration of the original formulation, providing more energy to sustain rapid cell growth and higher metabolic activity.

**33.** (D) RPMI-1640
RPMI-1640 medium was specifically developed to support the growth of human lymphocytes and other hematopoietic cells. It contains unique components like glutathione and high levels of vitamins that cater to the specific nutritional needs of these cell types. Originally used for leukemic cells, it has become a standard choice for various suspension cultures and immunological research involving human cells.

**34.** (C) It grows cells without a $CO_2$ atmosphere
The specific advantage of Leibovitz's L-15 medium is that it allows cells to grow without a $CO_2$ atmosphere. It achieves this by using free-base amino acids, particularly galactose and sodium pyruvate, instead of the traditional bicarbonate buffering system. This makes it ideal for experiments where $CO_2$ control is unavailable or for transporting cells where maintenance of gas levels is difficult.

**35.** (B) Chick embryo extract
Chick embryo extract is a natural culture medium derived directly from biological sources. Unlike synthetic media such as DMEM or RPMI-1640, which are composed of precise chemical components, natural media like embryo extracts, plasma, or lymph contain a complex, undefined mixture of growth factors and nutrients. These are often used to supplement basal media or support fastidious tissue growth.

**36.** (D) Ham's $F_{12}$
Ham's $F_{12}$ medium was originally designed to support the cloning of Chinese Hamster Ovary cells at low densities. It is a complex, nutrient-rich formulation containing various trace elements and vitamins not found in simpler media. This composition makes it particularly effective for serum-free applications and for sustaining the growth of diverse cell types that require a more specialized nutritional profile.

**37.** (A) Calcium chloride
Calcium chloride provides divalent calcium ions that are critical for cellular adhesion in anchorage-dependent cells. These ions are necessary for the function of cell-surface proteins like cadherins and integrins, which mediate attachments between cells and to the culture substrate. Without adequate calcium, cells cannot properly adhere or spread, leading to poor growth and detachment from the culture vessel.

**38.** (C) Act as a buffering agent with $CO_2$
Sodium bicarbonate serves as a vital buffering agent in cell culture media. When used in a $CO_2$-enriched environment, it establishes a bicarbonate-$CO_2$ equilibrium that stabilizes the pH of the medium. This system is essential for neutralizing the acidic metabolic waste produced by growing cells, ensuring a physiological environment that supports continuous cell health, enzymatic activity, and efficient nutrient uptake.

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can be autoclaved, glassware can withstand dry heat, and air is effectively cleaned using HEPA filters. Proper sterilization ensures that biological activity is preserved while preventing contamination.

**20.** (B) III only
Ethylene oxide is a gas used to sterilize heat-sensitive equipment and plastics, but it is not used for liquid media because it is toxic and leaves residues. Standard sterilization practices include using HEPA filters for air and autoclaving heat-stable items at 121 degrees Celsius. Dry heat does require higher temperatures and longer times compared to moist heat methods.

**21.** (B) 7.2 - 7.4
The optimal pH range for most mammalian cell cultures is between 7.2 and 7.4. Maintaining this physiological range is crucial for cellular enzymes to function correctly and for overall cell health. Deviations from this range can lead to reduced growth rates, altered metabolism, or cell death. Buffers are added to the media to stabilize the pH against changes.

**22.** (C) Bicarbonate-carbon dioxide
The bicarbonate-carbon dioxide buffering system is the most common method for maintaining pH in cell culture incubators. Sodium bicarbonate in the media interacts with the $CO_2$ gas in the incubator atmosphere to create a stable equilibrium. This mimics the natural buffering system found in blood, providing a physiological environment that supports the growth and health of mammalian cells.

**23.** (B) 15 - 25°C
Teleost fish cell cultures typically require an incubation temperature range of 15 to 25 degrees Celsius. Unlike mammalian cells, which are adapted to internal body temperatures around 37 degrees, fish cells are derived from cold-blooded organisms. Maintaining these lower temperatures is essential for their metabolic processes and survival, reflecting the natural environmental conditions of the species being studied.

**24.** (B) 280 - 320 mOsm/kg
The standard osmolarity range for mammalian cell culture media is 280 to 320 mOsm/kg. This range is designed to be isotonic with the internal environment of the cells, preventing excessive swelling or shrinking due to osmotic pressure. Proper osmolarity is critical for maintaining cell volume, membrane integrity, and the correct concentration of intracellular solutes necessary for metabolic functions.

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**25.** (B) pH indicator
Phenol red acts primarily as a pH indicator in cell culture media. It allows researchers to visually monitor the acidity or alkalinity of the environment. As cells grow and metabolize, they produce lactic acid, which lowers the pH and changes the color of the media. This visual cue is vital for determining when the medium needs to be replaced.

**26.** (C) Yellow
Phenol red becomes yellow when the culture media becomes acidic, indicating a low pH. This change occurs as cells consume nutrients and release acidic metabolic byproducts like lactic acid. The shift from red to yellow serves as a clear visual signal to the researcher that the medium is exhausted and needs to be replaced to maintain healthy cell growth.

**27.** (D) Buffer pH outside a $CO_2$ incubator
HEPES is a zwitterionic buffering agent added to culture media to maintain pH stability outside of a $CO_2$ incubator. Unlike bicarbonate buffers, which require a specific $CO_2$ atmosphere to function, HEPES provides effective buffering across a range of physiological conditions. This is particularly useful during cell manipulation, observation, or transport where controlled $CO_2$ levels are not easily maintained.

**28.** (A) Lower osmolarity requirements
Fish cell culture media often requires lower osmolarity compared to mammalian media. This adaptation reflects the different physiological environments of aquatic organisms. While mammalian cells thrive in higher salt concentrations, fish cells, especially those from freshwater species, require a more dilute environment. Maintaining the correct osmotic balance is essential for preventing cellular stress and ensuring successful growth in laboratory cultures.

**29.** (B) Glucose
Glucose is the primary energy source in basal media for cell culture. It is a hexose sugar that cells readily metabolize through glycolysis and

tissues, significantly advancing virology and the production of life-saving vaccines against various infectious diseases.

**10.** (A) I and II
The L cell line was established by Wilton Earle, representing one of the first continuous cell lines from mouse connective tissue. Peyton Rous isolated the first tumor-causing virus, known as the Rous sarcoma virus. While Harry Eagle developed essential media, he did not develop monoclonal antibodies; that was achieved by Kohler and Milstein. Thus, statements I and II.

**11.** (D) Membrane filtration
Membrane filtration is the most appropriate method for sterilizing heat-labile culture media. Components like vitamins, growth factors, and proteins are sensitive to high temperatures and would degrade during autoclaving. Using filters with extremely small pores allows the liquid to pass through while physically removing bacteria and fungi, preserving the chemical integrity and biological activity of the media components.

**12.** (A) 0.22 μm
A standard pore size of 0.22 micrometers is used for filter-sterilizing culture media to effectively remove bacteria. This size is small enough to trap common bacterial contaminants while allowing liquid nutrients and proteins to pass through. It is the industry standard for ensuring sterility in biological solutions that cannot withstand the high temperatures of autoclaving or dry heat.

**13.** (C) 121°C at 15 psi
Routine autoclaving is performed at 121 degrees Celsius and 15 psi of pressure. This specific combination of moist heat and pressure ensures the destruction of all microbial life, including highly resistant bacterial endospores. The process typically lasts for 15 to 20 minutes, making it an efficient and reliable method for sterilizing heat-stable laboratory equipment and specific types of media.

**14.** (B) Glassware
Glassware is best sterilized using dry heat in an oven. This method involves exposure to high temperatures, typically 160 to 180 degrees Celsius, for several hours. Unlike plastic or liquid media, glass can withstand intense dry heat without melting or degrading. This process effectively kills microbes by oxidizing cellular components, ensuring that laboratory vessels are sterile before use.

**15.** (A) Protein coagulation via moist heat
The sterilization mechanism of an autoclave relies primarily on protein coagulation via moist heat. High-pressure steam transfers heat more effectively than dry air, leading to the rapid denaturation and aggregation of essential microbial proteins and enzymes. This irreversible damage effectively kills bacteria, viruses, and spores, providing a high level of sterility for laboratory supplies that are moisture resistant.

**16.** (C) Forming thymine dimers in DNA
UV irradiation in biological safety cabinets sterilizes surfaces by forming thymine dimers in the DNA of microorganisms. These dimers disrupt the DNA structure, preventing successful replication and transcription. This damage ultimately leads to the death of bacteria and viruses on exposed surfaces. UV light is an effective germicide for maintaining sterility in the workspace between active procedures.

**17.** (D) Gamma irradiation
Gamma irradiation causes the least structural damage to empty plastic petri dishes while ensuring complete sterilization. This method uses ionizing radiation to disrupt microbial DNA without applying the high heat that would melt or warp plastic materials. It is widely used by manufacturers to provide pre-sterilized, ready-to-use plasticware for laboratory applications, maintaining the physical integrity and clarity.

**18.** (C) Heat degrades essential growth factors
Filtration is preferred for sterilizing fetal bovine serum because heat degrades essential growth factors and proteins. Serum contains a complex mixture of hormones and nutrients vital for cell proliferation that are sensitive to high temperatures. By using fine membrane filters, researchers can remove microbial contaminants without compromising the serum's ability to support healthy cell growth and maintain culture viability.

**19.** (B) Liquid antibiotics - Autoclave
Autoclaving is incorrect for liquid antibiotics because most are heat-labile and will lose their potency at high temperatures. Instead, these solutions must be sterilized using membrane filtration. In contrast, metal tools

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**39.** (A) Thiamine
Thiamine, also known as vitamin $B_1$, is a key constituent of standard basal media. It acts as a coenzyme in essential metabolic pathways, including the decarboxylation of alpha-keto acids. This process is crucial for energy production and the synthesis of cellular components. Including thiamine ensures that cultured cells can effectively process nutrients to sustain their growth, maintenance, and reproductive functions.

**40.** (A) Both A and R are true and R is the correct explanation of A.
L-glutamine must be supplemented frequently because it is chemically unstable in liquid media. It spontaneously undergoes a deamination reaction, converting into ammonia and glutamic acid. Ammonia is a toxic metabolic byproduct that can inhibit cell growth and reduce viability if it accumulates. Regular supplementation ensures a constant supply of this essential amino acid while minimizing the impact of toxic degradation.

**41.** (B) To supply growth factors and hormones
The primary role of Fetal Bovine Serum in cell culture is to supply a complex mixture of growth factors, hormones, and attachment factors. These biological components stimulate cell proliferation, support differentiation, and help cells adhere to the culture surface. Serum also provides essential trace elements and proteins that act as buffers and detoxifiers, creating a supportive environment for diverse cells.

**42.** (C) Albumin
Albumin is the most abundant protein in serum and functions to bind and neutralize heavy metals, fatty acids, and various toxins. By sequestering these potentially harmful substances, albumin protects cells from oxidative stress and chemical damage. Additionally, it contributes to the osmotic pressure of the medium and serves as a carrier for hormones and other small molecules essential for growth.

**43.** (D) To destroy complement system proteins
Heat inactivation is performed on serum to destroy complement system proteins. These proteins are part of the innate immune response and can cause cell lysis or interfere with specific immunological assays. By heating the serum to 56 degrees Celsius for 30 minutes, these heat-sensitive proteins are denatured, making the serum safer for use in cultures where complement-mediated interference must be avoided.

**44.** (A) Fibronectin
Fibronectin is an important attachment factor abundantly present in Fetal Bovine Serum. It acts as a molecular bridge between the cell surface and the culture substrate, facilitating the adherence and spreading of anchorage-dependent cells. This interaction is mediated by integrin receptors on the cell membrane, which recognize specific sequences in fibronectin, promoting the signaling pathways necessary for survival.

**45.** (B) Batch-to-batch variability
A major disadvantage of using undefined serum is batch-to-batch variability. Because serum is a biological product, its exact composition of growth factors and nutrients can differ significantly between different lots. This inconsistency can lead to unpredictable results in experiments and makes it difficult to standardize culture conditions. Researchers must often test several batches to find one that supports their cells.

**46.** (B) 5 - 10%
Routine mammalian cell culture typically utilizes serum at a concentration range of 5 to 10%. This amount provides enough growth factors, hormones, and proteins to support robust cell proliferation and maintenance for most cell lines. While some fastidious cells may require higher levels, and others can be adapted to lower concentrations, the 10% supplement is the standard for general work.

**47.** (A) Insulin, Transferrin, Selenium
In chemically defined media, ITS stands for Insulin, Transferrin, and Selenium. This supplement is added to replace essential components typically provided by serum. Insulin promotes glucose uptake and metabolism, transferrin facilitates iron transport, and selenium acts as an antioxidant cofactor. Together, these components support cell survival and growth in a controlled, serum-free environment, reducing variability and contamination risks.

**48.** (C) Mediates iron transport
Transferrin plays a specific role in serum-free media by mediating the transport of iron into cells. Iron is an essential cofactor for many

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(A) 5,000 cells/mL
(B) 50,000 cells/mL
(C) 500,000 cells/mL
(D) 5,000,000 cells/mL

2012
**273.** The primary reason for changing culture media is to:
(A) Induce cellular transformation
(B) Replenish nutrients and remove toxic metabolic byproducts
(C) Synchronize the cell cycle
(D) Prevent genetic mutations

2002
**274.** Conditioned medium is often used to help grow fastidious or low-density cells because it contains:
(A) Secreted autocrine growth factors
(B) High levels of fresh glucose
(C) Aminopterin
(D) Toxic metabolites

2006
**275.** In hollow-fiber bioreactors, the molecular weight cut-off (MWCO) of the fibers determines:
(A) The speed of the cells
(B) The temperature of the system
(C) Which secreted products or nutrients can cross the fiber membrane
(D) The oxygen concentration

2019
**276.** What is a "spinner flask" primarily used for?
(A) Growing strictly adherent cells without microcarriers
(B) Cryopreserving cells
(C) Performing karyotyping
(D) Maintaining small-scale suspension cultures with continuous magnetic agitation

2010
**277.** Pluronic F-68 is a non-ionic surfactant added to suspension cultures to:
(A) Protect cells from shear stress and bubble damage
(B) Dissolve lipids
(C) Induce cell fusion
(D) Act as an antibiotic

2025
**278.** The "plating efficiency" of a cell line is defined as:
(A) The percentage of cells that survive freezing
(B) The time it takes for the population to double
(C) The percentage of seeded cells that attach and form colonies
(D) The amount of protein secreted per cell

2003
**279.** An increase in the doubling time of a cell culture generally indicates:
(A) Suboptimal conditions, senescence, or contamination
(B) Optimal growth conditions
(C) High levels of fresh serum
(D) Successful immortalization

2022
**280.** Identify the INCORRECT statement regarding cell culture media.
(A) BME is less complex than DMEM
(B) Glutamine is stable in liquid media for years
(C) Phenol red is toxic at very high concentrations
(D) $CO_2$ interacts with bicarbonate to form a buffer

2007
**281.** The use of antibiotics in primary culture isolation is:
(A) Strictly forbidden
(B) Only necessary for plant cells
(C) Useful to promote cell division
(D) Common practice due to the high risk of contamination from the host tissue

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2025
**282.** During mechanical disaggregation, cell viability is often lower than enzymatic disaggregation because:
(A) Enzymes protect the cell membrane
(B) Mechanical methods require toxic chemicals
(C) Tearing tissues physically crushes and lyses many cells
(D) It takes much longer

2023
**283.** The optimal pH for trypsin activity during cell dissociation is:
(A) 5.0 - 5.5
(B) 6.0 - 6.5
(C) 7.6 - 8.0
(D) 9.5 - 10.0

2023
**284.** Which chelator is often combined with trypsin to enhance cell detachment?
(A) EGTA
(B) EDTA
(C) Citrate
(D) HEPES

**58.** (C) Minimizes damage and preserves viability
Cold trypsinization is often preferred because it minimizes cellular damage and preserves higher viability. In this method, the tissue is soaked in trypsin at a low temperature, allowing the enzyme to penetrate the tissue without actively digesting it. The actual dissociation occurs when the temperature is raised, resulting in a gentler release of cells compared to continuous warm incubation.

**59.** (D) They mimic the in vivo tissue characteristics
Primary cultures are valuable because they closely mimic the characteristics and behavior of the original in vivo tissue. Unlike immortalized cell lines, they maintain their natural genetic profile and physiological responses. However, they have a limited proliferative lifespan and eventually undergo senescence. This authenticity makes them ideal for studying normal cell biology, drug toxicity, and tissue-specific functions in a controlled environment.

**60.** (A) Mincing, Washing, Enzyme incubation, Centrifugation, Resuspension
The correct sequence for enzymatic tissue dissociation starts with mincing the tissue into small pieces to increase surface area. The pieces are then washed to remove debris and red blood cells. Next, they are incubated with enzymes to break down the matrix. Centrifugation is used to pellet the released cells, which are finally resuspended in growth media to start culture.

**61.** (B) Hayflick limit
The Hayflick limit refers to the observation that normal primary somatic cells can only undergo a finite number of divisions before they stop growing. This limitation is a natural programmed process related to the shortening of telomeres during each round of DNA replication. Once the limit is reached, cells enter a non-dividing state called senescence, effectively preventing unlimited proliferation in vitro.

**62.** (B) Log phase
The log phase, or exponential phase, is characterized by rapid and constant cell proliferation. During this period, the cell population doubles at a regular interval as the cells have adapted to their environment and nutrients are plentiful. This is the stage when cells are most metabolically active and healthy, making it the ideal time for experiments and for harvesting cells.

**63.** (A) Stop dividing when they reach confluency
Contact inhibition causes normal cultured cells to stop dividing once they form a continuous monolayer and reach confluency. When cells physically touch one another, signaling pathways are activated that halt the cell cycle. This regulatory mechanism ensures that cells grow in an organized fashion and prevents the uncontrolled overgrowth often seen in transformed or cancerous cells that lose this property.

**64.** (C) Cell attachment and adaptation without division
The lag phase is the initial period after seeding where cells attach to the substrate and adapt to their new environment. During this time, there is no increase in cell number as the cells are synthesizing necessary proteins and recovering from the stress of subculturing. The duration of this phase depends on the cell type, seeding density, and provided media.

**65.** (D) Telomere shortening
Senescence in normal mammalian cell cultures is primarily linked to telomere shortening. Each time a cell divides, the protective caps at the ends of chromosomes become slightly shorter because DNA polymerase cannot fully replicate the very tips. Eventually, the telomeres become critically short, triggering a DNA damage response that permanently arrests the cell cycle, leading to the finite lifespan.

**66.** (C) Late log (exponential) phase
Subculturing or passaging is most appropriate during the late log phase, just before the cells reach full confluency. At this stage, the cells are in their most healthy and proliferative state, ensuring high viability upon transfer to new vessels. Waiting until the stationary phase can lead to nutrient depletion and toxic buildup, which may negatively impact the success of passage.

**67.** (B) A solid, non-toxic substrate for attachment
Anchorage-dependent cells must attach to a solid, non-toxic substrate to survive and proliferate. In the laboratory, this is typically provided by the surface of plastic flasks or dishes that have been treated to be hydrophilic. Without

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lactic acid and other metabolic wastes by the bacteria. The turbidity, or cloudiness, is caused by the high concentration of bacterial cells in the liquid. These obvious changes usually appear very rapidly.

**145.** (A) Fuzzy, multicellular mycelial growth floating on the medium
Fungal contamination, particularly mold, is easily characterized by fuzzy, multicellular mycelial growth floating on or in the medium. These colonies are often white, green, or black and are large enough to be seen with the naked eye. Under the microscope, they appear as a network of thin, branching filaments called hyphae, making them distinct from bacterial or cellular structures.

**146.** (C) Working with multiple cell lines simultaneously
A common source of cross-contamination in laminar flow hoods is working with multiple cell lines simultaneously. If a researcher uses the same pipette tips or handles different flasks at the same time, cells from one line can easily be transferred to another. Because some cell lines grow much faster than others, a few contaminating cells can quickly overgrow original cultures.

**147.** (A) Penicillin and Streptomycin
The combination of Penicillin and Streptomycin is the historical standard for preventing bacterial contamination in basic cell culture. Penicillin targets Gram-positive bacteria by interfering with cell wall synthesis, while Streptomycin inhibits Gram-negative bacteria by disrupting protein synthesis. Often abbreviated as Pen-Strep, this mixture is added to growth media to provide a broad spectrum of protection against common laboratory contaminants.

**148.** (D) It masks low-level contamination and promotes resistance
Routine use of prophylactic antibiotics is discouraged because it can mask low-level contamination and promote the development of resistant bacterial strains. Furthermore, some antibiotics can be toxic to the cultured cells themselves or interfere with their metabolism. It is better to rely on strict aseptic techniques and only use antibiotics when necessary, such as during the initial isolation of cells.

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**149.** (B) Cytopathic effects (CPE)
Viral contamination of animal cell cultures can sometimes be detected by observing cytopathic effects. These effects include visible changes to the host cells, such as swelling, shrinking, rounding up, or the formation of large, multinucleated syncytia. In some cases, the cells may detach from the surface and lyse. While not all viruses cause CPE, these are important indicators.

**150.** (A) P-1, Q-2, R-3, S-4
Different contaminants show unique characteristics: bacteria cause turbid, acidic media with visible rods or cocci, while yeast appear as ovoid particles that may show budding. Mold forms fuzzy, macroscopic colonies with branching hyphae. Mycoplasma is invisible under a light microscope but significantly alters cell metabolism. Recognizing these distinct signs is crucial for effective monitoring and maintaining sterile laboratory conditions.

**151.** (D) Dead cells with damaged membranes take up the dye
The Trypan blue dye exclusion test is based on the fact that dead cells with damaged membranes take up the dye, while live cells with intact membranes exclude it. Because the dye cannot cross a healthy cell membrane, live cells remain clear. This simple and rapid method allows researchers to estimate the proportion of viable cells in a suspension using a hemocytometer.

**152.** (C) Blue
When viewed under a microscope, dead cells treated with Trypan blue appear blue. The dye penetrates the compromised membranes of dead or dying cells and stains the cytoplasm and nucleus. In contrast, viable cells remain clear and shiny because their intact membranes prevent the dye from entering. This clear color difference makes it easy to count and calculate sample viability.

**153.** (B) Cellular metabolic activity / Viability
The MTT assay is used primarily to measure cellular metabolic activity, which serves as an indicator of viability and proliferation. It is a colorimetric assay that relies on the ability of living cells to reduce a tetrazolium salt. This method is widely used in drug screening and cytotoxicity studies to determine how different treatments affect the overall health and growth.

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transformed cultures. In healthy cultures, cells stop dividing when they touch each other. Transformed cells ignore these signals and continue to proliferate, resulting in dense, multilayered clumps. This behavior is a key indicator of malignant transformation and is used to identify potentially cancerous cells.

**78.** (B) They are highly heterogeneous and exactly mimic in vivo conditions
An incorrect statement about continuous cell lines is that they exactly mimic in vivo conditions. In reality, these cells are often highly heterogeneous and have undergone significant genetic and phenotypic changes during immortalization and prolonged culture. While they are easier to maintain and grow faster than primary cells, their abnormal karyotypes mean they may not always represent normal physiology accurately.

**79.** (A) Chinese Hamster Ovary
CHO stands for Chinese Hamster Ovary cells, which are a widely used continuous cell line in biotechnology. These cells are particularly valued for their ability to grow in large-scale cultures and for their efficiency in producing recombinant proteins. Their well-characterized genetics and adaptability to various media make them the industry standard for manufacturing therapeutic antibodies and other complex biological drugs.

**80.** (C) HEK-293 - Human endothelial keratinocytes
The incorrect pair is HEK-293, which stands for Human Embryonic Kidney cells, not human endothelial keratinocytes. These cells were immortalized by the insertion of adenovirus DNA and are commonly used in biology. In contrast, Vero cells are from monkey kidneys, BHK-21 from baby hamster kidneys, and Jurkat cells are a well-known line of human T lymphocytes used in immunology.

**81.** (D) Preserves the 3D tissue architecture and cellular interactions
Organ culture differs from cell culture because it preserves the three-dimensional tissue architecture and natural cellular interactions. Instead of breaking the tissue down into single cells, small pieces of the organ are maintained in a way that retains histological structure. This allows researchers to study complex physiological processes and tissue responses that cannot be captured in simplified cell monolayers.

**82.** (C) Raft or grid method
The raft or grid method is classically used to maintain tissue explants at the liquid-gas interface in organ culture. The tissue is placed on a support, such as a metal grid or a raft of filter paper, which floats on the surface of the medium. This setup ensures that the cells receive nutrients from below while having direct access to oxygen.

**83.** (B) Lack of nutrient and gas diffusion to the center of the tissue
A major limitation of long-term organ culture is the lack of nutrient and gas diffusion to the center of the tissue. Without a functional circulatory system to transport oxygen and nutrients, the core of the organ piece often becomes necrotic as waste products accumulate. This restricts the size of the tissue that can be cultured and limits the duration.

**84.** (D) A layer of agarose or filter paper
In the raft method, the tissue is typically supported by a layer of agarose or filter paper. This support material helps to hold the tissue at the surface of the liquid medium, facilitating optimal gas exchange while allowing nutrients to diffuse into the sample. This configuration is essential for maintaining the viability and structural integrity of the organ explant during study.

**85.** (A) Developmental biology and tissue physiology
Organotypic cultures are primarily valuable for studying developmental biology and tissue physiology. By preserving the spatial arrangement and interactions between different cell types, these cultures provide a more realistic model of how organs function and develop. They are used to investigate tissue maturation, morphogenesis, and the effects of hormones or drugs in a context that resembles the environment.

**86.** (C) Histological architecture
The histological architecture is generally lost during the transition from organ culture to conventional cell culture. In cell culture, tissues are dissociated into individual cells that grow as a monolayer or in suspension, destroying the original 3D structure. Organ culture aims to maintain this complex spatial organization, which is crucial for understanding how cells behave within a tissue context.

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this attachment, these cells cannot initiate the necessary signaling pathways for growth and will eventually undergo a specialized form of programmed cell death.

**68.** (D) Mitotic index
The mitotic index drastically decreases as a cell culture enters the stationary phase. This parameter represents the proportion of cells undergoing active division. In the stationary phase, the rate of new cell production slows down and eventually equals the rate of cell death due to nutrient depletion, accumulation of waste, and physical crowding, resulting in a plateau in number.

**69.** (A) Contact inhibition
Cellular transformation in vitro generally leads to the loss of contact inhibition. Unlike normal cells, transformed cells continue to divide even after they have formed a complete monolayer, leading them to pile up and form three-dimensional clumps or foci. This uncontrolled growth is a hallmark of malignancy and is often accompanied by anchorage independence and the ability to grow in suspension.

**70.** (A) IV, III, I, II
A typical batch cell culture growth curve follows a specific sequence of phases. It starts with the lag phase (IV), where cells adapt. This is followed by the log phase (III), where they proliferate exponentially. Next is the stationary phase (I), where growth plateaus. Finally, the decline or death phase (II) occurs as nutrients are exhausted and toxic waste products accumulate.

**71.** (A) Have bypassed the Hayflick limit and are immortal
Continuous cell lines are distinct because they have bypassed the Hayflick limit, allowing them to divide indefinitely. These immortal cells often arise from tumors or through artificial transformation with viruses. Unlike primary cells, they can be passaged many times without undergoing senescence, providing a consistent and renewable source of biological material for long-term research and large-scale manufacturing of proteins.

**72.** (D) Telomerase
Telomerase is the enzyme typically reactivated or overexpressed in immortalized continuous cell lines. It functions to maintain the length of telomeres by adding repetitive DNA sequences to the ends of chromosomes. By preventing telomere shortening during division, telomerase allows cells to escape the natural Hayflick limit and achieve immortality, a key characteristic of both cancer cells and established cell lines.

**73.** (B) Anchorage independence
Anchorage independence is a classic characteristic of transformed or tumor cell lines. Unlike normal cells, which must attach to a solid surface to grow, transformed cells can survive and proliferate while suspended in a liquid medium or semi-solid agar. This property is closely linked to their ability to invade other tissues and metastasize in a living organism, reflecting their malignancy.

**74.** (C) Cervical adenocarcinoma
The HeLa cell line was originally established from human cervical adenocarcinoma tissue. These cells were taken from a patient named Henrietta Lacks in 1951 without her knowledge. HeLa cells are famous for being the first human cells to be successfully immortalized in culture. Their aggressive growth and durability have made them a cornerstone of modern medical research for decades.

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**75.** (D) Mouse
Spontaneous immortalization in vitro occurs most frequently in cells derived from mice. While human and avian cells rarely become immortal without specific intervention, rodent cells often undergo genetic changes during culture that allow them to bypass senescence. This high rate of spontaneous transformation has led to the establishment of many mouse cell lines used extensively in immunology, genetics, and cancer research.

**76.** (A) SV40 (Simian Virus 40)
Viral transformation commonly utilizes Simian Virus 40 (SV40) to create continuous cell lines. The large T-antigen of SV40 binds to and inactivates key tumor suppressor proteins like p53 and pRb, which normally regulate the cell cycle. By neutralizing these proteins, the virus forces the cells into a state of continuous division, effectively immortalizing them for use in long-term studies.

**77.** (B) Loss of contact inhibition
The loss of contact inhibition is the term that describes cells piling up to form a focus in

the bottom of the vessel where they can stick together and suffer from poor nutrient and oxygen access. Constant stirring or shaking keeps the cells evenly distributed throughout the medium, ensuring that every cell receives the components necessary for growth.

**97.** (D) Poly-L-lysine
Poly-L-lysine is routinely used to coat plastics to enhance the attachment of fastidious cells. This positively charged synthetic polymer improves the electrostatic interaction between the negatively charged cell membrane and the culture surface. By creating a more favorable environment for adherence, it helps sensitive or poorly adherent cells to spread and grow more effectively on standard tissue culture treated plasticware.

**98.** (C) Tissue-culture (TC) plasma treatment
Modern cell culture flasks undergo tissue-culture plasma treatment to make their surfaces hydrophilic. This process uses an atmospheric plasma to introduce oxygen-containing functional groups onto the polystyrene plastic. These groups increase the surface energy, allowing the medium to wet the surface and providing a better environment for proteins and cells to attach, which is essential for adherent cell growth.

**99.** (A) 0.1 - 2 rpm
The ideal speed for a roller bottle apparatus is typically between 0.1 and 2 rpm. This slow rotation ensures that the cells remain attached to the inner surface while providing gentle, continuous movement of the medium. This low speed is enough to facilitate gas exchange and nutrient distribution without creating excessive shear stress that could detach or damage the growing cells.

**100.** (A) P-4, Q-2, R-1, S-3
Different culture vessels are optimized for specific tasks: T-flasks are used for routine stationary monolayer maintenance, while roller bottles provide high surface area through slow rotation. Spinner flasks support suspension cultures using magnetic stirring to keep cells moving. Multi-well plates are designed for high-throughput screening assays, allowing researchers to test many different conditions or samples simultaneously in labs.

**101.** (A) Airlift bioreactor
An airlift bioreactor uses gas sparging for mixing instead of a mechanical impeller. In this system, air or a gas mixture is bubbled into the bottom of a central draft tube, creating a density difference that drives the circulation of the media. This design is gentler than mechanical stirring, making it ideal for fragile animal cells that are shear sensitive.

**102.** (C) Baffles
In a stirred-tank bioreactor, baffles are installed on the internal walls to prevent the formation of a deep vortex and improve mixing efficiency. These vertical plates disrupt the circular flow created by the impeller, turning it into a more turbulent and effective mixing pattern. This ensures that oxygen and nutrients are distributed evenly throughout the culture while preventing the entrapment of air.

**103.** (D) Microcarriers
Scale-up of anchorage-dependent cells most commonly utilizes microcarriers. These are small beads that provide a large total surface area for cells to attach to while being suspended in a stirred medium. This approach allows adherent cells to be grown in large volumes using traditional bioreactor technology, significantly increasing the yield of cells or their secreted products compared to stationary methods.

**104.** (C) Dissolved oxygen (DO)
Dissolved oxygen is a highly critical parameter that is constantly monitored during large-scale bioreactor runs. Cells require a steady supply of oxygen for aerobic respiration, but its solubility in media is low. Monitoring ensures that the aeration and agitation systems are providing enough oxygen to meet the metabolic demands of a growing population without reaching levels causing stress.

**105.** (B) Generate lower shear stress, protecting fragile cells
Airlift bioreactors are generally preferred for animal cells because they generate lower shear stress, protecting fragile cells from damage. Unlike stirred-tank reactors that use mechanical impellers, airlift systems use gas bubbles for circulation, which is gentler on the delicate cell membranes. This reduced physical stress leads to higher cell viability and better production of biological products in large-scale cultures.

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**106.** (D) Pluronic F-68 or anti-foaming agents
Foaming in sparged bioreactors is mitigated by adding Pluronic F-68 or specific anti-foaming agents. Foam can trap cells and lead to death, as well as clog gas exit filters. Pluronic F-68 not only helps reduce foam but also protects cell membranes from the physical damage caused by bursting bubbles. These additives are essential for maintaining a stable environment.

**107.** (D) 10:1 or greater
The height-to-diameter ratio in an airlift bioreactor is typically 10:1 or greater. This tall, narrow design is necessary to create a sufficient pressure head and a long path for the rising gas bubbles, which ensures efficient circulation and oxygen transfer. The high aspect ratio also helps to maintain a consistent flow pattern, which is crucial for uniform mixing throughout.

**108.** (A) Continuous culture (Chemostat)
Continuous culture, also known as a chemostat, involves the continuous addition of fresh medium and the simultaneous removal of spent medium containing cells. This operation keeps the culture in a constant state of exponential growth by maintaining a steady concentration of nutrients and removing metabolic waste. It is a powerful tool for studying cell physiology under stable, long-term environmental conditions.

**109.** (C) Prolongs the productive stationary/decline phase
Fed-batch culture is advantageous because it prolongs the productive stationary or decline phase. By periodically adding concentrated nutrients to the bioreactor, researchers can prevent nutrient exhaustion and keep the cells alive and producing for a longer period than in a simple batch culture. This results in much higher concentrations of the desired product in the final harvest of cells.

**110.** (A) I and II
Stirred-tank bioreactors rely on mechanical impellers for mixing, while airlift systems utilize gas bubbles to circulate the media. These two methods represent the primary ways large-scale cultures are agitated. It is important to note that animal cells are generally much more sensitive to physical shear stress than bacterial cells, requiring gentler mixing strategies. Therefore, statements I and II are correct.

**111.** (B) Dextran, gelatin, or glass
Microcarriers are small beads typically made of materials like dextran, gelatin, or glass. These materials are chosen for their biocompatibility and ability to support cell attachment. By providing a surface for anchorage-dependent cells to grow on while being suspended in a stirred-tank bioreactor, microcarriers enable the large-scale production of cells that would otherwise require massive amounts of stationary surface area.

**112.** (D) They provide high surface area to volume ratios
The specific advantage of using microcarriers in scale-up is that they provide extremely high surface area to volume ratios. Because the beads are small and numerous, they offer a vast total area for cell attachment within a relatively small amount of liquid medium. This allows for very high cell densities in a bioreactor, maximizing productivity while minimizing the space required.

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**113.** (A) In the extracapillary space surrounding the fibers
In a hollow-fiber bioreactor system, cells usually grow in the extracapillary space surrounding the fibers. The fibers act like artificial blood vessels, carrying nutrient-rich medium through their interior. Nutrients and oxygen diffuse out through the porous fiber walls to the cells, while metabolic waste products diffuse back into the medium to be carried away, mimicking the natural microenvironment of tissues.

**114.** (C) Continuous supply of medium with simultaneous retention of cells
Perfusion culture systems are defined by a continuous supply of fresh medium with the simultaneous retention of cells within the bioreactor. Unlike continuous culture where cells are removed, perfusion keeps the cells inside, allowing them to reach very high densities. This constant exchange of media removes toxic byproducts and provides a steady stream of nutrients, supporting high productivity for durations.

**115.** (A) Capillary blood vessels
Hollow-fiber systems uniquely mimic the structure and function of capillary blood vessels. The semi-permeable fibers allow for the efficient exchange of small molecules like nutrients and oxygen while acting as a barrier for larger proteins and

the cells themselves. This arrangement creates a high-density, low-shear environment that closely resembles the physiological conditions found in solid tissues in the body.

**116.** (D) Spin filters or acoustic settlers
Cell retention in perfusion bioreactors is commonly achieved using specialized devices like spin filters or acoustic settlers. These systems allow spent medium to be removed from the reactor while keeping the cells inside. Spin filters use centrifugal force and physical barriers, while acoustic settlers use sound waves to aggregate cells so they sink back into the culture, enabling high densities.

**117.** (B) 100 - 300 μm
The standard diameter range for typical microcarrier beads is 100 to 300 micrometers. This size is large enough to provide a stable surface for hundreds of individual cells to attach and grow, yet small enough to be easily kept in suspension by gentle agitation in a bioreactor. This optimal size ensures a high surface-area-to-volume ratio, which is essential for efficiency.

**118.** (B) Hollow-fiber perfusion
Hollow-fiber perfusion systems yield the highest volumetric cell density compared to other methods. Because the cells are confined in a small space and continuously supplied with nutrients while waste is removed, they can reach concentrations exceeding 100 million cells per milliliter. This density is far greater than what can be achieved in standard T-flasks, roller bottles, or stirred-tank cultures.

**119.** (D) Difficulty in scale-up and monitoring specific cell masses
A major disadvantage of hollow-fiber systems is the difficulty in scale-up and monitoring the specific cell mass. Because the cells are packed tightly in the extracapillary space, it is hard to obtain accurate counts or observe their health directly. Furthermore, scaling up requires increasing the number of fiber units rather than just enlarging a single vessel, making it complex.

**120.** (B) II only
Perfusion systems are designed to retain cells while replacing media, and microcarriers require gentle agitation to keep them in suspension. Cytodex is a well-known brand of microcarrier. However, the statement that hollow-fiber culture provides very low cell densities is incorrect; in fact, it is known for providing exceptionally high cell densities. Therefore, statement II is the only incorrect one.

**121.** (A) Cross-contamination with other cell lines (e.g., HeLa)
Cell line authentication is critical to rule out cross-contamination with other cell lines. A common and serious problem in research is the inadvertent replacement of a specialized cell line with a faster-growing one, like HeLa. If left undetected, this contamination can invalidate experimental results and lead to the publication of incorrect data. Regular testing ensures researchers work with correct materials.

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**122.** (C) Short Tandem Repeat (STR) profiling
Short Tandem Repeat (STR) profiling is the current gold standard for human cell line authentication. This molecular technique analyzes specific regions of the genome that vary in length between individuals. By creating a unique genetic fingerprint, researchers can compare their cell line against established databases to confirm its identity and ensure that no cross-contamination has occurred, providing confidence in results.

**123.** (D) Chromosomal stability and number
Karyotyping is primarily used to evaluate chromosomal stability and number in a cell line. By staining and observing chromosomes during metaphase, scientists can identify large-scale genetic abnormalities such as aneuploidy, translocations, or deletions. This characterization is essential because many continuous cell lines develop abnormal genomes over time, which can significantly alter their behavior and the outcomes of experiments performed.

**124.** (C) Species of origin
Isoenzyme analysis distinguishes cell lines primarily based on their species of origin. Different species have unique electrophoretic patterns for specific enzymes, such as lactate dehydrogenase. By running a cell lysate on a gel and staining for these enzymes, researchers can confirm if a cell line is from a human, mouse, rat, or other animal. This is a vital check.

**125.** (B) Bipolar or multipolar, elongated cells
Fibroblasts in culture typically exhibit a classic morphology characterized by bipolar or

multipolar, elongated cells. They often appear spindle-shaped and grow in an organized, parallel fashion when they become confluent. This appearance reflects their role in producing connective tissue components in the body. Identifying these features under a microscope is a basic yet important step in confirming cell population nature.

**126.** (D) Giemsa
G-banding in chromosomal karyotyping typically utilizes Giemsa stain. This technique involves treating chromosomes with an enzyme like trypsin followed by staining, which creates a pattern of dark and light bands. These bands are unique to each chromosome and allow for the precise identification of individual pairs. This detailed analysis is essential for detecting structural changes and ensuring the genetic integrity of lines.

**127.** (A) Polygonal patches or sheets
Epithelial cells in culture typically display a characteristic morphology, forming polygonal patches or continuous sheets. These cells grow closely together with tight junctions, reflecting their natural role as barrier tissues in the body. Unlike spindle-shaped fibroblasts, epithelial cells have a more cuboidal or cobblestone appearance under the microscope, which is a key identifying feature used by researchers to monitor cultures.

**128.** (C) It is contaminated with human cells
If a presumed murine cell line shows a human LDH isoenzyme band, it is contaminated with human cells. Each species has a distinct electrophoretic pattern for the lactate dehydrogenase enzyme. Finding a band that corresponds to a different species is a definitive sign of cross-contamination. This result indicates that the culture is no longer a pure murine population and should be discarded.

**129.** (B) Microsatellite DNA
STR profiling relies on amplifying specific regions of microsatellite DNA. These short tandem repeats are non-coding sequences that vary significantly in length between different individuals. By using PCR to amplify these regions and measuring the resulting fragments, scientists can create a genetic profile that is unique to a specific cell line, allowing for highly accurate identification and the detection of contamination.

**130.** (A) I and III
STR profiling is a highly specific method for identifying human cell lines, and karyotyping is a powerful tool for observing metaphase chromosomes to check for genetic stability. Isoenzyme analysis is indeed effective at differentiating species. However, HeLa contamination remains a major contemporary problem, and morphological checks alone are insufficient for formal authentication. Thus, statements I and III are correct.

**131.** (D) The initial cloned or established cell population
The Master Cell Bank is derived directly from the initial cloned or established cell population. It consists of a large number of identical vials that are frozen and stored for long-term use. The MCB serves as the ultimate source for all future cultures, ensuring that a consistent, well-characterized starting material is always available to replace working stocks and maintain production integrity.

**132.** (A) It is generated by expanding one or more vials from the MCB
The fundamental rule regarding the Working Cell Bank is that it is generated by expanding one or more vials from the Master Cell Bank. The WCB is what researchers actually use for their daily experiments or production runs. By using a two-tiered banking system, the original MCB is protected from exhaustion and contamination, ensuring that the cell line remains available.

**133.** (B) Dimethyl sulfoxide (DMSO)
Dimethyl sulfoxide (DMSO) is the most commonly used cryoprotectant in cell banking. When added to the freezing medium, it penetrates the cell membrane and lowers the freezing point of water. This helps to prevent the formation of large, lethal ice crystals during the cooling process. DMSO is essential for maintaining high cell viability when thawing cells after storage in liquid nitrogen.

**134.** (B) Preventing intracellular ice crystal formation
DMSO prevents freeze-damage to cells primarily by preventing intracellular ice crystal formation. As the temperature drops, DMSO disrupts the hydrogen bonding between water molecules, encouraging the formation of a vitreous, glass-like state instead of sharp crystals that would rupture the cell membrane and organelles. This

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protective action is crucial for preserving the structural integrity and functionality of cells during storage.

**135.** (A) -1°C per minute
The optimal cooling rate for cryopreserving mammalian cells is generally -1 degree Celsius per minute. This slow, controlled rate allows water to leave the cell gradually, reducing the chance of internal ice formation while preventing excessive dehydration. This balance is critical for maximizing the survival rate of the cells upon thawing, as both internal ice and severe dehydration cause death.

**136.** (D) Mr. Frosty (isopropanol container)
Specialized containers like the Mr. Frosty ensure the correct controlled cooling rate during cryopreservation. These devices are filled with isopropanol and placed in a -80 degree Celsius freezer. The alcohol acts as a heat sink, ensuring that the temperature inside the vials drops steadily at approximately 1 degree per minute. This simple and effective method is standard for preparing cells.

**137.** (C) Rapid thawing in a 37°C water bath
The standard protocol for reviving frozen cells requires rapid thawing in a 37 degree Celsius water bath. Fast warming is essential because it prevents the recrystallization of small ice crystals into larger, damaging ones as the ice melts. Once the ice has just disappeared, the vial is immediately moved to a sterile hood for further processing to minimize toxicity.

**138.** (A) To remove toxic DMSO
Cells must be washed or diluted immediately after thawing to remove the toxic cryoprotectant, DMSO. While DMSO is necessary during the freezing process, it is harmful to living cells at room temperature or 37 degrees. By adding fresh medium and centrifuging the cells, researchers can replace the DMSO-containing liquid with healthy growth medium, ensuring the cells can recover properly.

**139.** (C) -196°C
Liquid nitrogen is used for long-term cell storage because it maintains an extremely low temperature of -196 degrees Celsius. At this temperature, all biological activity and metabolic processes effectively stop, allowing cells to remain viable for many years or even decades. Storing vials in the vapor phase above the liquid is often preferred to prevent the accidental entry of liquid nitrogen.

**140.** (A) Both A and R are true and R is the correct explanation of A.
Cells must be frozen slowly to minimize intracellular ice formation, but they must be thawed rapidly to prevent the recrystallization of water. If thawing is slow, small ice crystals can grow and merge, creating large structures that pierce the cell membranes and destroy the cell. Rapid thawing ensures a quick transition to a liquid state, preserving recovered population viability.

**141.** (D) Mycoplasma
Mycoplasma is the most insidious contaminant in cell culture because it is too small to be seen with a light microscope and does not cause obvious turbidity. These tiny organisms can persist in a culture for a long time, subtly altering cell growth, metabolism, and gene expression without the researcher's knowledge, potentially ruining experimental data and conclusions drawn from them.

**142.** (C) They lack cell walls and are very small
Mycoplasma contamination is often invisible because these organisms lack a cell wall and are extremely small, typically ranging from 0.15 to 0.3 micrometers. Their small size places them below the resolution limit of standard light microscopes. Furthermore, they do not cause rapid cell death or significant changes in medium color, allowing them to spread undetected throughout a laboratory's cultures.

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**143.** (B) Hoechst 33258
Hoechst 33258 is a fluorescent dye frequently used for the rapid visual detection of Mycoplasma. This dye binds specifically to DNA, and when viewed under a fluorescence microscope, contaminated cultures will show small, bright particulate matter outside the host cell nucleus. This simple staining method allows researchers to quickly screen their cultures for the presence of these difficult-to-detect and harmful contaminants.

**144.** (D) Turn yellow (acidic) and become turbid
Bacterial contamination typically causes the culture medium to turn yellow and become turbid. The yellow color indicates an acidic pH shift, resulting from the rapid production of

**87.** (A) Morphogen and hormone gradients
Embryonic organ culture is highly dependent on the precise regulation of morphogen and hormone gradients. These chemical signals guide the differentiation and spatial organization of cells during development. In an in vitro environment, researchers must carefully control the concentration and distribution of these factors to ensure that the cultured tissue develops correctly and accurately reflects natural embryonic processes.

**88.** (D) Diffusion through 3D tissue mass is highly restricted
Gas exchange is more critical in organ culture because diffusion through the three-dimensional tissue mass is highly restricted. Unlike a thin monolayer where every cell is in direct contact with the media and air, organ explants consist of many layers. Oxygen must travel deeper into the tissue, and metabolic $CO_2$ must escape, making efficient exchange vital for survival.

**89.** (B) Air-liquid interface (ALI) culture
Air-liquid interface culture simulates an in vivo-like microenvironment for 3D organ culture by exposing the top of the cells to air while the bottom is submerged in nutrient media. This technique is especially useful for modeling epithelial tissues like the skin or respiratory tract, as it encourages natural differentiation and the formation of protective barriers not present in submerged cultures.

**90.** (A) Both A and R are true and R is the correct explanation of A.
Organ cultures cannot be expanded by passaging because the process involves breaking the tissue into single cells, which destroys the intricate 3D structural organization. The value of organ culture lies in maintaining these complex intercellular relationships and histological architecture. Consequently, once the tissue is dissociated for subculturing, it transitions into a standard cell culture, losing unique organ model properties.

**91.** (D) Available surface area for cell attachment in $cm^2$
T-flasks are named based on their available surface area for cell attachment, measured in square centimeters. For example, a T-25 flask has 25 $cm^2$ of growth surface, while a T-75 has 75 $cm^2$. This naming convention allows researchers to easily calculate seeding densities and scale experiments appropriately, ensuring that anchorage-dependent cells have sufficient space to adhere, spread, and proliferate.

**92.** (B) 75 $cm^2$
A T-75 flask provides approximately 75 $cm^2$ of surface area for cell growth. This standardized size is commonly used in laboratory research for maintaining and expanding adherent cell lines. The specific surface area is designed to support a predictable number of cells at confluency, allowing scientists to maintain consistent experimental conditions and manage the growth of their cultures with precision in labs.

**93.** (B) Greatly increase available surface area for anchorage-dependent cells
Roller bottle culture systems are designed to greatly increase the available surface area for anchorage-dependent cells. By slowly rotating the bottles, cells can grow on the entire inner surface, which is much larger than the base of a stationary flask. This method allows for higher cell yields in a relatively compact space, making it a popular choice for production.

**94.** (B) Better aeration and continuous mixing of media
A key advantage of roller bottles is better aeration and continuous mixing of the media. As the bottle rotates, the cells are alternately exposed to the nutrient medium and the gas phase in the bottle. This process ensures a steady supply of oxygen and prevents the localized depletion of nutrients, supporting healthier and more productive cultures compared to stationary flasks.

**95.** (A) Hematopoietic and lymphoid cells
Suspension cultures are most appropriate for growing hematopoietic and lymphoid cells, such as those found in blood or the immune system. These cells naturally exist as individual units in the body and do not require a solid surface for attachment to survive or divide. Growing them in suspension allows for easy scale-up and simplified harvesting since no dissociation is needed.

**96.** (C) Cell settling and clumping
Agitation in suspension cultures is crucial to prevent cell settling and clumping. Without continuous movement, cells tend to sink to

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**154.** (D) Insoluble purple formazan crystals
In the MTT assay, viable cells convert the soluble yellow MTT tetrazolium salt into insoluble purple formazan crystals. This conversion occurs inside the mitochondria of living cells. The intensity of the resulting purple color, measured after the crystals are dissolved in a solvent, is directly proportional to the number of metabolically active cells in the sample, providing a quantitative measure.

**155.** (A) Mitochondrial succinate dehydrogenase
The enzyme responsible for the conversion of MTT to formazan is mitochondrial succinate dehydrogenase. This enzyme is a part of the respiratory chain in active mitochondria. Because only living, metabolically active cells possess functional mitochondria, the production of the purple product is a reliable marker for cell viability. The assay effectively measures the reducing capacity of the tested cell population.

**156.** (C) The formazan product is water-soluble, skipping the solubilization step
The XTT assay offers the advantage that its formazan product is water-soluble, which allows researchers to skip the final solubilization step required in the classic MTT assay. After the incubation period, the absorbance can be read directly from the culture plate. This simplification saves time and reduces potential errors associated with the addition of organic solvents like DMSO or isopropanol.

**157.** (A) Loss of membrane integrity and cytotoxicity
The release of Lactate Dehydrogenase into the culture medium is a classic marker for the loss of membrane integrity and cytotoxicity. LDH is a stable cytosolic enzyme that is only released when the cell membrane is damaged or ruptured. By measuring the amount of LDH in the media, researchers can quantify the extent of cell death caused by experimental treatments.

**158.** (D) Dead cells with compromised membranes
Propidium Iodide specifically stains dead cells with compromised membranes. It is a fluorescent dye that binds to DNA but cannot cross the membrane of a healthy, living cell. When the membrane is damaged during death or late-stage apoptosis, PI enters the cell and stains the nucleus bright red. This property makes it a powerful tool for identifying dead cells.

**159.** (B) Intracellular esterases in live cells
Calcein-AM is used in viability assays because it fluoresces green only when cleaved by intracellular esterases in live cells. The AM group makes the molecule membrane-permeant, allowing it to enter cells freely. Once inside, non-specific esterases remove the AM group, trapping the now-fluorescent calcein inside the cell. Because these enzymes are only active in living cells, the green signal marks viability.

**160.** (A) Seed cells → Add drug → Incubate → Add MTT → Solubilize → Read Absorbance
The correct sequence for an MTT assay begins with seeding the cells into a plate, followed by adding the experimental drug and incubating. After the treatment period, MTT is added and incubated to allow formazan crystals to form. These crystals are then solubilized with a solvent, and finally, the absorbance of the solution is measured using a plate reader.

**161.** (D) Mononuclear cells (lymphocytes and monocytes) from whole blood
Density gradient centrifugation using Ficoll-Hypaque is widely used to isolate mononuclear cells, such as lymphocytes and monocytes, from whole blood. This technique separates cells based on their specific density. During centrifugation, the red blood cells and granulocytes sink through the Ficoll layer, while the lighter mononuclear cells remain at the interface, allowing for their clean collection and research use.

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**162.** (B) 1.077 g/mL
The specific gravity of standard Ficoll-Hypaque used for human PBMC isolation is 1.077 g/mL. This density is carefully calibrated to be between the density of lymphocytes and the density of red blood cells. By layering blood over this solution and centrifuging, the heavier cells pass through the gradient while the lighter mononuclear cells are trapped on top, facilitating separation.

**163.** (A) At the buffy coat interface between the plasma and Ficoll
After proper centrifugation, the lymphocytes are found at the buffy coat interface between the plasma and the Ficoll layer. Because their density is lower than the Ficoll-Hypaque solution, they cannot pass through it and instead form a visible, cloudy white layer. This interface can be carefully aspirated with a pipette, providing a

hand, epitope mapping is an analytical tool that does not alter antibody structure, and IgG is the most common isotype for monoclonal antibodies.

**221.** (B) CD3
$OKT_3$ was the first FDA-approved therapeutic monoclonal antibody, designed to target the CD3 receptor on T cells. By binding to this receptor, the antibody prevents T-cell activation and effectively suppresses the immune response that leads to organ transplant rejection. This pioneering drug demonstrated the immense potential of monoclonal antibodies as targeted therapies, paving the way for numerous antibody-based treatments.

**222.** (A) CD20
Rituximab is a monoclonal antibody used in cancer immunotherapy that targets the CD20 antigen found on the surface of B cells. By binding to CD20, it triggers the destruction of these cells through various mechanisms, including complement-mediated lysis and antibody-dependent cellular cytotoxicity. It is used to treat B-cell lymphomas and certain autoimmune diseases by depleting the malignant B-cell population.

**223.** (C) Antibody-drug conjugates (ADCs)
Antibody-drug conjugates (ADCs) consist of a monoclonal antibody linked to a radioactive isotope or a potent cytotoxic drug. This technology uses the high specificity of the antibody to deliver the toxic payload directly to target cells, such as cancer cells, while sparing healthy tissues. ADCs increase the efficacy of treatment and reduce systemic side effects, representing a significant therapeutic advancement.

**224.** (D) HER2-positive breast cancer
Trastuzumab is an engineered monoclonal antibody used specifically to treat HER2-positive breast cancer. It binds to the human epidermal growth factor receptor 2, which is overexpressed in some aggressive types of breast cancer. By blocking the signaling pathways that promote cell growth and survival, Trastuzumab helps to inhibit tumor progression and improve survival rates for patients with this genetic profile.

**225.** (C) Human chorionic gonadotropin (hCG)
Home pregnancy tests utilize monoclonal antibodies to detect human chorionic gonadotropin (hCG) in urine. hCG is a hormone produced by the placenta shortly after fertilization and its presence is a reliable indicator of pregnancy. The antibodies in the test strip bind specifically to hCG, triggering a color change that provides a fast and highly accurate result in diagnostic testing.

**226.** (B) Blood group typing (ABO)
Blood group typing is a classic example of an in vitro diagnostic use of monoclonal antibodies. By using antibodies specific to A, B, or Rh antigens, clinicians can quickly and accurately determine a person's blood type. This application relies on the high specificity and consistent performance of MAbs, ensuring safe blood transfusions and providing essential information for various medical procedures.

**227.** (D) PD-1, $PD\text{-}L_1$, or CTLA-4
Checkpoint inhibitors are a breakthrough in cancer therapy that utilize monoclonal antibodies to target proteins like PD-1, $PD\text{-}L_1$, or CTLA-4. These proteins normally act as brakes on the immune system to prevent overactivity. By blocking these checkpoints, the antibodies release the brakes, allowing the patient's own T cells to recognize and attack tumor cells more effectively for improvement.

**228.** (A) Autoimmune and inflammatory diseases
Anti-TNF-alpha monoclonal antibodies, such as Infliximab, are primarily used to treat autoimmune and inflammatory diseases like rheumatoid arthritis and Crohn's disease. TNF-alpha is a pro-inflammatory cytokine that plays a central role in these conditions. By neutralizing this molecule, these antibodies reduce inflammation, alleviate symptoms, and prevent further tissue damage, greatly improving the quality of life for patients.

**229.** (C) They trigger a systemic HAMA response
A significant drawback of using pure murine monoclonal antibodies in humans is that they trigger a systemic HAMA (Human Anti-Mouse Antibody) response. Because the human immune system recognizes the mouse proteins as foreign, it produces antibodies against them. This response can lead to rapid clearance of the drug from the body, reduced therapeutic effectiveness, and potentially serious allergic reactions.

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viral and host membranes. By using inactivated Sendai virus, researchers can trigger the fusion of two different animal cells to create heterokaryons or hybridomas without the risk of viral replication or infection.

**174.** (A) Short, high-intensity electric pulses to cause membrane breakdown
Electrofusion utilizes short, high-intensity electric pulses to cause a temporary breakdown of the cell membrane, a process called electroporation. When cells are held in close contact, these microscopic pores allow the membranes to merge as they reseal. This method is highly controlled and efficient, providing a cleaner alternative to chemical fusion methods like PEG, with typically lower toxicity.

**175.** (D) Heterokaryon
A heterokaryon is the resulting fused cell that contains two or more distinct nuclei from different parent cells before they merge into a single nucleus. This state is temporary, as the nuclei will eventually fuse during the next round of cell division to form a true hybrid cell. Heterokaryons are useful for studying the interactions between different cytoplasmic and nuclear components.

**176.** (B) It offers higher fusion efficiency and lower cellular toxicity
The primary advantage of electrofusion over PEG fusion is that it offers higher fusion efficiency and significantly lower cellular toxicity. While PEG is a harsh chemical that can damage cell membranes if not removed quickly, electrofusion uses precise electrical pulses that are less harmful. This results in a higher yield of viable hybrid cells, making it a preferred method.

**177.** (C) A whole cell fused with an enucleated cytoplast
A cybrid, or cytoplasmic hybrid, is a unique fusion product consisting of a whole cell fused with an enucleated cytoplast. This creates a cell that contains the nucleus of one parent and the cytoplasm, including the mitochondria, of another. Cybrids are powerful tools for studying the role of mitochondrial DNA and cytoplasmic factors in cell behavior, inheritance, and various diseases.

**178.** (A) Align the cells into "pearl chains" prior to the fusion pulse
Dielectrophoresis is used in electrofusion to align the cells into pearl chains prior to the application of the fusion pulse. By using a high-frequency alternating current, cells are polarized and drawn together at the points of highest field strength. This close physical contact is essential for the subsequent high-voltage pulse to successfully trigger the merging of the adjacent cell membranes.

**179.** (D) UV irradiation to inactivate its replication while preserving fusion capability
Sendai virus for cell fusion is usually prepared by UV irradiation to inactivate its replication while preserving its ability to fuse membranes. The UV light damages the viral genome, preventing it from multiplying inside the host cells. However, the viral envelope proteins remain functional, allowing the virus to act as a bridge and catalyst for merging the membranes of different cells.

**180.** (B) It is highly toxic to cells upon prolonged exposure
A significant disadvantage of using Polyethylene Glycol (PEG) is that it is highly toxic to cells upon prolonged exposure. The chemical process that allows membranes to fuse also stresses the cells, leading to low viability if the PEG is not rapidly washed away after the fusion event. Despite being inexpensive and simple to use, this toxicity limits overall efficiency.

**181.** (B) Aminopterin
In somatic cell genetics, the de novo synthesis pathway of nucleotides can be blocked by aminopterin. This compound is a folic acid analogue that inhibits the enzyme dihydrofolate reductase, which is essential for the synthesis of purines and thymidylate. By shutting down this pathway, aminopterin forces cells to rely on alternative salvage pathways to obtain the nucleotides necessary for survival.

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**182.** (D) Hypoxanthine-guanine phosphoribosyltransferase (HGPRT)
HGPRT is the essential enzyme for the purine salvage pathway that allows cells to survive when the de novo pathway is blocked by aminopterin. HGPRT enables the cell to utilize exogenous hypoxanthine to produce the necessary purine nucleotides. In selection systems like HAT, only

direct contact with a tumor cell. It consists of two linked single-chain variable fragments, one targeting a tumor-specific antigen and the other targeting the CD3 receptor on T cells. This arrangement bypasses normal activation pathways, forcing T cells to kill the cancer cells.

**240.** (A) Both A and R are true and R is the correct explanation of A.
Chimeric antibodies are less likely to induce a HAMA response than pure murine antibodies because they contain human constant regions. The constant regions make up the largest part of the antibody and are highly immunogenic if they are from a different species. By humanizing these regions, researchers significantly reduce the risk of the drug being cleared or causing reactions.

**241.** (D) VH and VL regions joined by a peptide linker
A single-chain variable fragment (scFv) is composed of the variable heavy (VH) and variable light (VL) regions of an antibody, joined together by a short peptide linker. This small, recombinant protein maintains the full antigen-binding specificity of the original antibody but lacks the constant regions. Its compact size and simplicity make it a versatile tool for diagnostic imaging.

**242.** (C) They are small enough to easily penetrate dense solid tumors
A primary advantage of scFv molecules is that they are small enough to easily penetrate dense solid tumors. Unlike full-sized antibodies, which can have difficulty reaching the center of a large tissue mass, the compact structure of scFvs allows them to move more freely through the extracellular matrix. This improved penetration can lead to better therapeutic outcomes and diagnostic imaging.

**243.** (B) Expressed on the surface coat of the bacteriophage
In phage display technology, the gene encoding the antibody fragment is inserted into the phage genome so that the fragment is expressed on the surface coat of the bacteriophage. This creates a physical link between the genotype and phenotype. By screening large libraries of these phages, researchers can identify specific binders to any target antigen, facilitating new antibody discovery.

**244.** (D) Iterative biopanning of phage display libraries
Biopanning is the process of isolating phages through repeated cycles of binding to a target, washing away non-binders, and amplifying the selected phages in bacteria. This iterative process gradually enriches the population for phages that display high-affinity antibody fragments. Biopanning is the core procedure in phage display technology, allowing for the rapid identification of human antibodies without experimental animals.

**245.** (A) Escherichia coli
Escherichia coli is the microorganism typically used to host and amplify the bacteriophages in phage display libraries. E. coli is easy to grow and manipulate, making it an ideal factory for producing large quantities of the selected phages between rounds of biopanning. Its well-understood genetics also facilitate the cloning and expression of the antibody genes discovered during screening.

**246.** (C) Transfected CHO cells or E. coli
Recombinant antibodies can be rapidly mass-produced in completely serum-free systems using transfected CHO cells or E. coli. These systems allow for high-level expression of antibody genes in a controlled, animal-free environment. This approach ensures high purity, consistency, and safety of the final product, which is essential for biological drugs used in human patients, while being more scalable than traditional methods.

**247.** (A) Two scFvs linked together
A diabody is a recombinant antibody format consisting of two scFvs linked together. This design creates a bivalent molecule that can bind two antigens simultaneously. Because the linkers are too short for the regions to fold back on themselves, they are forced to pair with the regions of another chain, resulting in a stable dimer with increased binding strength.

**248.** (D) Light chains
Camels and llamas produce a unique class of functional antibodies that naturally lack light chains. These heavy-chain-only antibodies use a single variable domain to recognize their target. Because they are smaller and more stable than traditional antibodies, they have become an important area of research. These nanobodies

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generation. This specific inbred strain is chosen because most standard myeloma fusion partners are also derived from BALB/c mice. This genetic compatibility ensures that the resulting hybridomas can be grown as tumors in mice to produce high concentrations of antibodies, known as ascites production.

**193.** (C) HGPRT-deficient myeloma cell lines
Sp2/0 and NS-1 are common examples of HGPRT-deficient myeloma cell lines used in hybridoma technology. These immortal cells serve as the fusion partners for antibody-producing B cells. They are carefully selected for their ability to grow rapidly in culture and for their lack of the salvage enzyme, which allows for the efficient selection of successful hybrids using HAT medium.

**194.** (D) Be non-secretory for endogenous antibodies
A crucial feature of an ideal myeloma partner is that it should be non-secretory for endogenous antibodies. If the myeloma cell produced its own heavy or light chains, the resulting hybridoma would secrete a mixture of antibodies, making the purification of the target monoclonal antibody much more difficult. Using a non-secretory line ensures all antibodies produced are antigen specific.

**195.** (C) 5:1 to 10:1
The typical recommended ratio for PEG fusion is 5:1 to 10:1 of spleen cells to myeloma cells. This excess of spleen cells increases the probability that each myeloma cell will come into contact with and fuse with a B cell. Since unfused myelomas are eliminated by HAT selection, this ratio helps to maximize the number of antibody-producing hybridomas generated.

**196.** (C) Stimulate antigen-specific B cell proliferation
The booster immunization administered 3 days before cell fusion is designed to stimulate the rapid proliferation of antigen-specific B cells. This final injection ensures that a large population of active, antibody-secreting lymphoblasts is present in the spleen at harvest. By maximizing the number of relevant B cells, researchers significantly increase the chances of successfully creating hybridomas that produce desired antibodies.

**197.** (A) Supply growth factors and improve survival at low cell densities
Feeder cells are often added to supply essential growth factors and improve the survival of newly fused hybridomas at low cell densities. After fusion, hybridoma cells are often fragile and plated into many small wells, where they may lack sufficient autocrine signaling. Macrophages provide a supportive microenvironment, releasing cytokines that encourage the hybrids to survive, attach, and begin dividing.

**198.** (D) They are derived from multiple B-cell clones recognizing different epitopes
Polyclonal antibodies are derived from multiple B-cell clones that recognize different epitopes on the same antigen. This broad recognition is characteristic of the natural immune response. While polyclonal serums are useful for some applications, they lack the high specificity and batch-to-batch consistency of monoclonal antibodies, which are produced by a single clone and recognize only one specific site.

**199.** (B) It drastically enhances the immune response to the injected antigen
An adjuvant, like Freund's Complete Adjuvant, drastically enhances the immune response to the injected antigen. It works by creating a depot that releases the antigen slowly and by stimulating the innate immune system, leading to better recruitment and activation of B and T cells. This results in higher titers of specific antibodies, increasing the success rate of hybridoma fusions.

**200.** (A) P-3, Q-1, R-4, S-2
In hybridoma technology, the spleen B-cell provides specific antibody secretion, while the myeloma cell provides immortality. Polyethylene glycol (PEG) is the chemical agent that induces membrane fusion between these two cell types. Aminopterin is the component in HAT medium that blocks the de novo synthesis pathway, allowing for the selective survival of successfully fused hybrid cells during initial culture.

**201.** (D) Sub-cloning
Sub-cloning is the process of isolating a single hybridoma cell from a mixed population to establish a uniform, monoclonal culture. This step is essential because initial fusion products often contain multiple cell types or unstable hybrids. By ensuring that every cell in the resulting colony

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cells that possess a functional HGPRT enzyme can survive and grow, making it a critical marker.

**183.** (A) Hypoxanthine and Thymidine are provided in the medium
Cells utilize the salvage pathway only when hypoxanthine and thymidine are provided in the medium and the de novo pathway is blocked. In normal conditions, cells prefer the de novo pathway for nucleotide synthesis. However, in the presence of aminopterin, they must switch to salvaging these external precursors from the environment to survive. This metabolic flexibility is exploited in selection.

**184.** (C) Hypoxanthine
In HAT selection, the H stands for Hypoxanthine. This compound is a purine derivative that serves as a precursor for the salvage pathway. When the de novo synthesis of nucleotides is blocked by aminopterin, cells must use hypoxanthine, along with the enzyme HGPRT, to produce the purines necessary for DNA and RNA synthesis. This ensures that only cells with the enzyme survive.

**185.** (A) Thymidine
A cell line that is deficient in thymidine kinase (TK-) cannot salvage thymidine from the medium. Like HGPRT, TK is a key enzyme in the salvage pathway, specifically for pyrimidine nucleotides. In selection systems where the de novo pathway is blocked, TK-deficient cells will die because they cannot produce the thymidylate required for DNA replication, even if thymidine is available.

**186.** (D) They lack the HGPRT enzyme for salvage
Unfused myeloma cells die in HAT medium because they lack the HGPRT enzyme required for the salvage pathway. These cells are specifically selected to be HGPRT-deficient before the fusion process. When aminopterin blocks their de novo nucleotide synthesis, they have no alternative way to produce the purines needed for survival. Consequently, only myeloma cells fused with spleen cells survive.

**187.** (B) They are primary cells with a limited lifespan and die naturally
Unfused splenic B-cells eventually die in HAT medium because they are primary cells with a limited natural lifespan. Although they possess the functional HGPRT and TK enzymes necessary to survive the aminopterin block, they are not immortal. Unlike the hybridoma cells, which inherit immortality from the myeloma parent, the normal spleen cells will naturally undergo senescence and die in culture.

**188.** (C) Spleen-myeloma hybrid cells (hybridomas)
Spleen-myeloma hybrid cells, or hybridomas, are the only cells that survive HAT selection. They inherit immortality from the myeloma parent and the functional HGPRT and TK salvage enzymes from the spleen cell parent. This combination allows them to grow indefinitely while bypassing the aminopterin block. Unfused myelomas die from the block, and unfused spleen cells die naturally in the media.

**189.** (D) HGPRT-
A mutant cell line that is resistant to the toxic analogue 8-azaguanine is usually HGPRT-deficient. 8-azaguanine is a guanine mimic that the HGPRT enzyme incorporates into DNA, leading to cell death. Cells that lack functional HGPRT cannot use this toxic compound and therefore survive its presence. This selection method is commonly used to isolate the HGPRT-negative myeloma lines for production.

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**190.** (A) Both A and R are true and R is the correct explanation of A.
HGPRT-deficient cells survive in normal media because they can use the de novo pathway for nucleotide synthesis. However, they die in HAT media because aminopterin blocks this pathway, and their lack of the HGPRT enzyme prevents them from using the salvage pathway. This metabolic requirement is the basis for HAT selection, ensuring only successfully fused hybrid cells proliferate.

**191.** (B) Monoclonal antibodies
Hybridoma technology specifically produces monoclonal antibodies. These are highly specific antibodies derived from a single clone of B cells, ensuring that all the antibody molecules in a batch are identical and recognize the same epitope on an antigen. This consistency makes them invaluable for diagnostic assays, therapeutic treatments, and basic research where precise targeting and reproducible results are essential.

**192.** (A) Mouse (typically BALB/c)
The BALB/c mouse is the animal most frequently immunized to provide spleen cells for hybridoma

**211.** (D) Isotyping
Isotyping is the process of determining the specific class and subclass of a monoclonal antibody. In mice, this typically involves identifying whether the antibody is IgG, IgM, IgA, or IgE, and specifically which IgG subclass it belongs to. This information is crucial for understanding the antibody's biological properties, such as its halF-life, complement activation, and suitable purification methods.

**212.** (C) IgG
Most murine monoclonal antibodies generated through standard hybridoma technology are of the IgG isotype. This class is favored because of its high affinity, stability, and ease of purification. Furthermore, the immunization protocols used generally select for the isotype switching that leads to IgG production. These antibodies are highly effective for research, diagnostic, and therapeutic applications due to their binding characteristics.

**213.** (A) Protein A or Protein G
Protein A or Protein G are bacterial proteins widely used for the affinity purification of IgG monoclonal antibodies. These proteins have a very high and specific affinity for the Fc region of the IgG molecule. By immobilizing them on a solid support, researchers can selectively capture antibodies from complex mixtures like culture supernatant, allowing for efficient, one-step purification with yield.

**214.** (D) The precise binding site of the antibody on the target antigen
Epitope mapping determines the precise binding site of an antibody on its target antigen. This process identifies the specific sequence of amino acids or the three-dimensional structure that the antibody recognizes. Understanding the epitope is vital for characterizing how the antibody functions, predicting potential cross-reactivity, and designing improved vaccines or therapeutic agents that target specific regions of a protein.

**215.** (A) Papain
Monoclonal antibodies can be cleaved into Fab and Fc fragments by the enzyme papain. Papain is a cysteine protease that cuts the antibody molecule above the hinge region, resulting in two separate, monovalent Fab fragments and one intact Fc fragment. This enzymatic digestion is used to study the individual functions of the binding regions and the effector portion in assays.

**216.** (C) One F(ab')2 fragment
Pepsin digestion of an IgG molecule primarily yields one F(ab')2 fragment and several small pieces of the Fc region. Unlike papain, pepsin cleaves the antibody below the hinge region, leaving the two antigen-binding sites connected by disulfide bonds. This results in a bivalent fragment that can still cross-link antigens but lacks the biological effector functions associated with the constant portion.

**217.** (A) Improved tissue penetration due to smaller size
The primary advantage of using a Fab fragment is improved tissue penetration due to its smaller size compared to a whole antibody. Because it lacks the Fc region, it is less likely to be trapped by non-specific interactions or trigger unwanted immune responses. This makes Fab fragments useful in imaging and for reaching targets in dense tissues where larger molecules are restricted.

**218.** (D) A low pH buffer (e.g., pH 2.5 - 3.0)
Affinity chromatography typically elutes the bound antibody using a low pH buffer, such as pH 2.5 to 3.0. The acidic environment disrupts the non-covalent interactions between the antibody and the immobilized ligand, such as Protein A. Once released from the column, the antibody is immediately neutralized with a higher pH buffer to prevent denaturation and maintain its biological activity.

**219.** (B) Lateral flow immunoassay or ELISA
Isotyping is rapidly performed using lateral flow immunoassays or specialized ELISA kits. These tests use antibodies specific for each immunoglobulin class and subclass to identify the isotype of the monoclonal antibody in a culture supernatant. This information is essential for choosing the correct purification method and for understanding the potential effector functions the antibody might possess in downstream research applications.

**220.** (A) I and II
Papain cleavage yields two monovalent Fab fragments and one Fc fragment, while Protein G is known for its strong binding to the Fc region of various IgG subclasses. These are fundamental facts in antibody characterization. On the other

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concentrated and relatively pure population of mononuclear cells.

**164.** (C) In the pellet at the bottom
Red blood cells and granulocytes end up in the pellet at the very bottom of the tube after Ficoll gradient centrifugation. These cells have a higher density than the 1.077 g/mL Ficoll-Hypaque solution, causing them to sediment through the gradient during the run. This separation effectively removes them from the lighter mononuclear cells, which remain at the interface above the layer.

**165.** (B) Mitogens
Mitogens are substances that induce mitosis and activate lymphocytes in culture. These molecules bypass the specific antigen receptor and trigger global cellular proliferation by binding to various surface receptors. Mitogens are essential tools in immunology for testing the functional capacity of immune cells and for expanding specific populations, such as T or B cells, for various laboratory experimental uses.

**166.** (D) T lymphocytes
Phytohemagglutinin (PHA) is a mitogen that primarily stimulates T lymphocytes to divide. Extracted from red kidney beans, PHA binds to the T-cell receptor complex, mimicking the signal for activation. This results in the rapid proliferation and transformation of resting T cells into large, active lymphoblasts. It is widely used in diagnostic tests to evaluate the cellular immune response of patients.

**167.** (A) Lipopolysaccharide (LPS)
Lipopolysaccharide (LPS), derived from the outer membrane of Gram-negative bacteria, is a B-cell mitogen in mice. It triggers the proliferation and differentiation of B lymphocytes independently of T-cell help. In laboratory experiments, LPS is used to study B-cell activation, antibody production, and the innate immune responses mediated by Toll-like receptors, providing insights into how the immune system recognizes bacterial threats.

**168.** (C) Tritiated thymidine ([3H]-TdR)
Lymphocyte proliferation is classically quantified by incorporating tritiated thymidine into the DNA of dividing cells. As the cells replicate their genome, they take up the radioactive thymidine from the culture medium. The amount of radioactivity measured in the harvested cells is directly proportional to the rate of DNA synthesis and, therefore, the degree of cellular proliferation induced by a mitogen.

**169.** (A) Jack bean
Concanavalin A (ConA) is a lectin derived from the Jack bean, Canavalia ensiformis. Like PHA, it is a potent T-cell mitogen that binds to carbohydrate groups on cell surface glycoproteins. ConA is extensively used in immunological research to induce cytokine production and study the mechanisms of T-cell activation and proliferation in both human and animal cell models in vitro.

**170.** (A) III, II, I, IV
The correct sequence for isolating PBMC begins with diluting the whole blood with PBS to reduce viscosity. The diluted blood is then carefully layered over the Ficoll-Hypaque solution. Next, the tube is centrifuged without using the brake to prevent disturbing the layers. Finally, the cloudy interface containing the mononuclear cells is collected with a pipette for further washing.

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**171.** (D) Microinjection
Microinjection is a cellular micromanipulation technique used to introduce DNA, proteins, or other molecules directly into a specific cell nucleus. Using an extremely fine glass needle and a high-precision micromanipulator, the researcher can bypass the cell membrane and deliver the material with high accuracy. While labor-intensive and limited to a few cells at a time, it ensures direct delivery.

**172.** (B) Dehydrating the cell surfaces and disrupting lipid bilayers
Polyethylene glycol (PEG) promotes cell fusion by dehydrating the cell surfaces and disrupting the lipid bilayers. When cells are brought into close contact in the presence of PEG, the water molecules surrounding the membranes are removed, causing the lipids to become disordered. This state allows the membranes of adjacent cells to merge, resulting in the formation of a single, multinucleated hybrid.

**173.** (C) Sendai virus
Sendai virus is classically used to induce fusion between mammalian cells. The virus possesses specialized envelope proteins, such as the fusion protein, that naturally mediate the merging of

**230.** (A) IV only
Rituximab targets CD20, Trastuzumab targets HER2, Bevacizumab targets VEGF, and Pembrolizumab targets PD-1, all of which are correct clinical associations. However, Infliximab is designed to target TNF-alpha, not CD3. CD3 is the target of $OKT_3$, which is used to prevent transplant rejection. Therefore, only the pair matching Infliximab with CD3 is incorrect in this list of antibodies.

**231.** (B) Human constant regions and murine variable regions
Chimeric antibodies were developed to reduce immunogenicity by combining human constant regions with murine variable regions. By replacing the majority of the mouse protein with human sequences, these antibodies are less likely to be recognized as foreign by the human immune system. This design maintains the original antigen specificity while improving safety and longevity for therapeutic use in human patients.

**232.** (A) -ximab
In standard nomenclature, a chimeric monoclonal antibody typically ends with the suffix -ximab. Examples include Rituximab and Infliximab. This suffix indicates that the antibody's structure is a hybrid, usually consisting of approximately 65% human protein. The naming convention helps clinicians and researchers quickly identify the degree of humanization and the potential risk for immunogenic reactions when administered to patients.

**233.** (C) Only the Complementarity-Determining Regions (CDRs) are murine
Humanized monoclonal antibodies are engineered so that only the Complementarity-Determining Regions (CDRs) are of murine origin. The rest of the antibody, including the constant regions and the framework of the variable regions, is human. This advanced engineering further reduces the likelihood of an immune response compared to chimeric antibodies, making them safer and more effective for therapeutic applications in humans.

**234.** (D) CDR grafting
CDR grafting is the process of transferring murine Complementarity-Determining Regions onto a human antibody framework. This technique allows researchers to preserve the specific antigen-binding site of a mouse antibody while replacing almost all other parts with human sequences. The result is a humanized antibody that maintains high affinity for its target while minimizing its foreignness, significantly reducing immune reactions.

**235.** (A) Using transgenic mice possessing human immunoglobulin genes
Fully human monoclonal antibodies can be generated by using transgenic mice that possess human immunoglobulin genes. When these mice are immunized, they produce human antibodies instead of mouse ones. These B cells can then be used in standard hybridoma technology to create immortal cell lines that secrete 100% human antibodies, completely eliminating the need for subsequent humanization in labs.

**236.** (C) ~90-95%
Approximately 90-95% of a humanized antibody, identified by the suffix -zumab, is typically of human origin. Only the small loops that directly contact the antigen are derived from the original mouse antibody. This high level of humanization is designed to ensure that the drug is well-tolerated by the human immune system, allowing for multiple doses over time safely.

**237.** (B) In vitro phage display library screening
In vitro phage display library screening is a strategy used to completely avoid animals in the generation of human antibodies. This technology involves displaying millions of different antibody fragments on the surface of bacteriophages. By selecting those that bind to a specific target, researchers can isolate human antibody sequences entirely in the laboratory. This method is fast and ethically sound.

**238.** (D) Bind to two completely different antigens or epitopes simultaneously
Bispecific antibodies are uniquely engineered to bind to two completely different antigens or epitopes simultaneously. This dual-targeting capability allows them to perform complex functions, such as bringing an immune cell into close contact with a tumor cell or blocking two different signaling pathways at once. This versatility makes them powerful tools for developing more effective cancer therapies for various diseases.

**239.** (A) Bispecific T-cell engager (BiTE)
A Bispecific T-cell engager (BiTE) is a designer antibody built to bring a cytotoxic T-cell into

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is identical, researchers guarantee the long-term production of a single, consistent antibody with defined specificity.

**202.** (A) Limiting dilution
Limiting dilution is the most universally used technique for sub-cloning hybridomas. This method involves serially diluting a cell suspension until there is a high probability of having only one cell per well in a multi-well plate. As these single cells grow into colonies, they form genetically identical populations. This simple approach ensures the clonality of the antibody-producing cell line.

**203.** (A) 0.5 to 1 cell per well
In limiting dilution, cells are diluted to a statistical target of 0.5 to 1 cell per well. This ensures that many wells will contain no cells, while those that do have a high likelihood of starting from a single progenitor. By following this distribution, researchers can be reasonably sure that any colony that develops is a true monoclonal cell clone.

**204.** (D) To ensure clonality and prevent overgrowth
Sub-cloning is performed early to ensure clonality and prevent the overgrowth of desired cells by faster-growing, non-secreting hybrids. Newly formed hybridomas are often genetically unstable and can lose the chromosomes responsible for antibody production. Rapidly isolating a productive clone ensures specific antibody-secreting capability is preserved and that the resulting cell line remains stable and productive for long-term use.

**205.** (A) Anchorage independence
Soft agar cloning relies on the anchorage independence of hybridoma cells. Like many transformed or cancerous cells, hybridomas can survive and form colonies while suspended in a semi-solid matrix. This method allows for the visual identification and physical isolation of individual colonies that have grown from single cells. It is an alternative to liquid-based limiting dilution for ensuring monoclonality.

**206.** (B) Enzyme-Linked Immunosorbent Assay (ELISA)
Enzyme-Linked Immunosorbent Assay (ELISA) is the standard method for high-throughput screening of hybridoma supernatants. It allows researchers to quickly test hundreds of samples for specific antibodies. The assay is highly sensitive, quantitative, and can be automated, making it the ideal choice for identifying which wells contain the desired hybridomas during the early stages of clone selection and subsequent characterization.

**207.** (A) The specific antigen of interest
In an indirect ELISA for screening, the microtiter plate is initially coated with the specific antigen of interest. The hybridoma supernatant is then added, allowing any target-specific antibodies to bind to the immobilized antigen. Subsequent steps involve adding an enzyme-linked secondary antibody and a substrate to produce a measurable signal, confirming the presence of the desired monoclonal antibody in wells.

**208.** (C) A cell-surface associated molecule
Flow cytometry can be used to screen hybridomas if the target antigen is a cell-surface associated molecule. In this application, the hybridoma supernatant is incubated with cells that express the antigen. If specific antibodies are present, they will bind to the cell surface and can be detected using fluorescently labeled secondary antibodies. This method identifies antibodies recognizing native protein conformations.

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**209.** (A) Development of a visible color change upon adding substrate
A positive well in a standard colorimetric ELISA is indicated by a visible color change upon adding the substrate. This change occurs because the enzyme linked to the secondary antibody reacts with the substrate, producing a colored product. The intensity of the color is proportional to the amount of specific monoclonal antibody that bound to the antigen, allowing for identification of productivity.

**210.** (B) I and III
Sub-cloning ensures the monoclonality of a hybridoma line and can be performed using various methods, including limiting dilution or flow cytometry-based cell sorting. While limiting dilution is common, the goal is to have less than one cell per well, not more than five. Therefore, statements I and III accurately describe the purpose and techniques used to isolate pure populations.

can reach epitopes that are hidden or inaccessible to larger molecules.

**249.** (B) Nanobody
The isolated variable domain of a heavy-chain-only antibody is commonly referred to as a nanobody. Despite their small size, nanobodies maintain high affinity and specificity for their targets. They are very stable, easy to produce in large quantities, and can be engineered into various formats. Their unique properties make them excellent candidates for new types of diagnostics and therapies for diseases.

**250.** (A) P-2, Q-4, R-3, S-1
Different antibody formats have distinct structures: IgG is a full Y-shaped molecule, while Fab is a fragment resulting from papain cleavage. A scFv consists of VH and VL regions connected by a peptide linker, and a nanobody is a single VHH domain from camelids. Matching these formats to their descriptions is essential for understanding the tools available in modern biotechnology.

**251.** (B) Volume of the $CO_2$ incubator
The volume of the $CO_2$ incubator is least likely to affect the success of primary cell isolation. Factors like the age of the animal, the type and concentration of enzymes used, and the time taken to process the tissue are far more critical. While an incubator provides the environment, its size does not directly impact the biological efficiency.

**252.** (D) Serve as an intermediate carbon/energy source bypass for glycolysis
Sodium pyruvate is added to serve as an intermediate carbon and energy source that can bypass the need for early steps in glycolysis. This is particularly useful for cells with low metabolic rates or those under stress. By providing a direct entry point into the citric acid cycle, pyruvate helps to maintain cellular energy levels and supports growth in culture.

**253.** (A) Alpha-1 antitrypsin
Alpha-1 antitrypsin is a serum-derived factor that specifically inhibits trypsin activity. In cell culture, serum is often added after trypsinization to stop the action of the enzyme and prevent it from damaging the cells. This protease inhibitor naturally occurring in the serum effectively neutralizes the trypsin, ensuring that the detached cells are protected and can begin the recovery process.

**254.** (C) To prevent the formation of damaging secondary ice crystals
Rapid immersion in a 37 degree Celsius water bath is recommended to prevent the formation of damaging secondary ice crystals as the cells thaw. If thawing is slow, small ice crystals can grow and merge, creating larger structures that can puncture cell membranes and lead to death. Fast warming ensures a quick transition to the liquid state, maximizing viability.

**255.** (A) The available attachment surface area
The maximum cell density achievable in a standard T-flask is ultimately limited by the available attachment surface area. Anchorage-dependent cells must adhere to a solid surface to grow, and once they form a complete monolayer, they stop dividing due to contact inhibition. While nutrients and gas exchange are important, the physical space for attachment is the primary constraint.

**256.** (D) HL-60 leukemia cells
HL-60 leukemia cells are inherently capable of growing in suspension without the need for microcarriers. As a hematopoietic cell line, they naturally exist as individual units and do not require a solid surface for attachment or survival. This property makes them easy to maintain in large-scale cultures using spinner flasks or stirred-tank bioreactors for various research applications in laboratories.

**257.** (B) An abnormal number of chromosomes
An aneuploid cell line is characterized by having an abnormal number of chromosomes. This condition is very common in continuous and transformed cell lines, where genetic instability leads to the gain or loss of individual chromosomes. Aneuploidy can significantly alter the phenotype and behavior of cells, which is why periodic karyotyping is essential to monitor the genetic health.

**258.** (C) Contact inhibition
Contact inhibition is the phenomenon where normal cells stop proliferating once they reach a high density and membranes touch. This process is a vital regulatory mechanism that ensures cells grow in an organized, single layer. When cells are crowded, signaling pathways are triggered that

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(D) I, II and III

2013
**8.** In Multiple Ovulation and Embryo Transfer (MOET), why is donor-recipient synchrony crucial?
(A) It aligns uterine and embryonic development stages
(B) It ensures the embryos hatch properly prior to transfer
(C) It prevents fertilization by multiple sperm in the donor
(D) It increases the overall ovulation rate within the recipient

2025
**9.** Which morphological feature is LEAST relevant when grading an embryo according to IETS guidelines?
(A) Degree of cellular fragmentation
(B) Color of the zona pellucida
(C) Uniformity of blastomeres
(D) Stage of development relative to age

2004
**10.** Analyze the given assertion and reason, then select the correct option:
**Assertion (A):** The presence of cumulus cells around the oocyte is critical for successful IVM.
**Reason (R):** Cumulus cells provide essential nutrients and regulatory signals through gap junctions.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2012
**11.** What causes the cortical reaction during fertilization?
(A) Calcium release upon sperm-oocyte fusion
(B) Binding of sperm to the ZP3 receptor
(C) Activation of protein kinase A in the sperm tail
(D) Secretion of progesterone by the cumulus cells

2005
**12.** Which statement correctly identifies an advantage of using defined media in embryo culture?
(A) It contains undefined biological components to boost embryo growth
(B) It requires mandatory co-culture with specific helper somatic cells
(C) It eliminates disease transmission risk from serum
(D) It completely prevents the need for strict temperature controls

2007
**13.** Which of the following is NOT a recognized technique for micro-assisted fertilization?
(A) Intracytoplasmic sperm injection
(B) Partial zona dissection
(C) Subzonal insemination
(D) Somatic cell nuclear transfer

2010
**14.** What principle allows flow cytometry to separate X and Y chromosome-bearing sperm?
(A) Differences in sperm tail length
(B) Differences in swimming speed
(C) Differences in total DNA content
(D) Differences in acrosome shape

2009
**15.** Consider the following statements about embryo sexing:
I. PCR-based sexing amplifies Y-chromosome specific sequences.
II. Karyotyping requires dividing cells to visualize chromosomes.
III. H-Y antigen assays are highly accurate and have replaced PCR.
IV. Biopsy of the blastocyst is generally required for DNA extraction.
Which of the above statements are correct?
(A) I, II and IV
(B) I, III and IV
(C) II, III and IV
(D) I, II and III

2025
**16.** In the context of cryobiology, what is the primary purpose of seeding during slow-freezing?
(A) To rapidly vitrify the complex intracellular contents
(B) To completely remove all cryoprotectants from the media
(C) To cause instantaneous and complete cellular dehydration
(D) To induce extracellular ice formation

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2003
**17.** Which is an INCORRECT pairing of a cryoprotective agent and its classification?
(A) Sucrose - Permeating
(B) Glycerol - Permeating
(C) Dimethyl sulfoxide - Permeating
(D) Trehalose - Non-permeating

reproducibility, it is critical to use cells within defined passage ranges.

**269.** (D) Quantifying cell concentration (cells/mL)
A hemocytometer is specifically used for quantifying cell concentration in a liquid suspension. This specialized microscope slide has a finely etched grid and a precise volume under the coverslip. By counting the number of cells in specific squares and using a mathematical formula, researchers can accurately calculate the total number of cells per milliliter, which is essential for seeding.

**270.** (A) Both A and R are true and R is the correct explanation of A.
Routine Mycoplasma testing is strongly recommended because these organisms grow slowly and do not cause visible turbidity in the medium. However, they can profoundly alter cell metabolism, signal transduction, and gene expression, potentially invalidating research results. Regular screening ensures that any contamination is caught early, protecting the integrity of the data and preventing the spread to other cell lines.

**271.** (A) 0.0001 mL ($10^{-4}$ mL)
When counting cells using a standard hemocytometer, the volume of one large corner square is 0.0001 mL. This precise volume is achieved by the specific dimensions of the square (1 mm by 1 mm) and the fixed height of the coverslip (0.1 mm). Knowing this volume is fundamental for calculating the cell concentration of the original cell suspension accurately.

**272.** (C) 500,000 cells/mL
If you count an average of 50 cells in a standard large square, the concentration is 500,000 cells/mL. This is calculated by multiplying the average cell count by the dilution factor and then by 10,000. Accurate cell counting is essential for seeding experiments at the correct density and for monitoring the growth rates of cultures over a period of time.

**273.** (B) Replenish nutrients and remove toxic metabolic byproducts
The primary reason for changing culture media is to replenish essential nutrients and remove toxic metabolic byproducts. As cells grow, they consume glucose, amino acids, and vitamins, while releasing waste products like lactic acid and ammonia. If the medium is not refreshed, the nutrient depletion and toxic buildup will inhibit growth and eventually lead to cell death in culture.

**274.** (A) Secreted autocrine growth factors
Conditioned medium is used because it contains secreted autocrine growth factors produced by cells that were previously grown in it. These factors can promote the survival and proliferation of fastidious or low-density cells that might otherwise struggle to grow in fresh, unconditioned media. This technique is often used during cloning or when establishing new cell lines to provide support.

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**275.** (C) Which secreted products or nutrients can cross the fiber membrane
In hollow-fiber bioreactors, the molecular weight cut-off (MWCO) of the fibers determines which secreted products or nutrients can cross the fiber membrane. A low MWCO allows small molecules like glucose and oxygen to reach the cells while keeping large proteins and the cells themselves trapped. This allows for the concentration of desired products in a small volume for harvesting.

**276.** (D) Maintaining small-scale suspension cultures with continuous magnetic agitation
A spinner flask is primarily used for maintaining small-scale suspension cultures with continuous magnetic agitation. The flask contains a hanging stir bar that keeps the cells evenly distributed in the medium, ensuring consistent access to nutrients and oxygen. This method is ideal for growing hematopoietic cells or for adapting adherent cells to suspension, providing a simple way to increase volume.

**277.** (A) Protect cells from shear stress and bubble damage
Pluronic F-68 is a non-ionic surfactant added to suspension cultures to protect cells from shear stress and bubble damage. In agitated bioreactors, the physical forces of stirring and the bursting of air bubbles can rupture delicate cell membranes. Pluronic F-68 stabilizes the membranes and reduces the surface tension, minimizing cell death and improving the overall health of the culture.

**278.** (C) The percentage of seeded cells that attach and form colonies
The plating efficiency of a cell line is defined as the percentage of seeded cells that attach to the surface and form viable colonies. This parameter is a measure of the cell line's ability to survive the stress of subculturing and grow at low densities. It is an important factor in cloning experiments and for assessing the overall quality.

**279.** (A) Suboptimal conditions, senescence, or contamination
An increase in the doubling time generally indicates suboptimal conditions, senescence, or contamination. The doubling time is the period it takes for a cell population to double in number. When cells are healthy and in an ideal environment, this time is consistent. A slowdown in growth suggests that the cells are stressed, aging, or competing with microbial contaminants.

**280.** (B) Glutamine is stable in liquid media for years
Glutamine is known to be chemically unstable in liquid media and will degrade over a period of weeks or months, not years. This degradation produces ammonia, which is toxic to cells. Therefore, it is standard practice to store glutamine-containing media for limited times or add it fresh. Other statements about the relative complexity of media and buffering are accurate.

**281.** (D) Common practice due to the high risk of contamination from the host tissue
The use of antibiotics in primary culture isolation is common practice due to the high risk of contamination from the host tissue. Unlike established cell lines grown in sterile conditions, tissues taken from organisms can harbor various bacteria and fungi. Adding broad-spectrum antibiotics to the initial media helps to eliminate these contaminants, giving the primary cells a better survival chance.

**282.** (C) Tearing tissues physically crushes and lyses many cells
During mechanical disaggregation, cell viability is often lower because the physical forces of tearing, mincing, or sieving can crush and lyses many individual cells. While enzymatic methods use chemical action to gently break down the extracellular matrix, mechanical methods rely on physical disruption, which is inherently harsher. This results in a higher proportion of damaged cells and laboratory debris.

**283.** (C) 7.6 - 8.0
The optimal pH for trypsin activity during cell dissociation is between 7.6 and 8.0. At this slightly alkaline pH, the enzyme is most effective at cleaving the proteins that hold cells to each other and to the culture surface. Maintaining the correct pH is important for ensuring rapid and complete detachment while minimizing the time cells are exposed to trypsin.

**284.** (B) EDTA
EDTA is often combined with trypsin to enhance cell detachment. As a chelator, EDTA binds to calcium and magnesium ions that are necessary for the function of cell adhesion molecules like cadherins. By removing these ions, EDTA weakens the bonds between cells, making it easier for trypsin to access and digest the protein bridges, resulting in a more efficient process.

**285.** (A) Proteolytic degradation of essential cell surface receptors
A disadvantage of using excessively high concentrations of trypsin is the proteolytic degradation of essential cell surface receptors. While trypsin is needed to detach cells, too much of it can begin to digest important proteins like growth factor receptors and adhesion molecules. This damage can slow down the cells' ability to recover and re-attach once they are transferred.

**286.** (C) Cells completely covering the available surface area with no empty spaces
A confluent monolayer represents the state where cells have completely covered the available surface area of the culture vessel, leaving no empty spaces between them. In this state, anchorage-dependent cells are in physical contact with their neighbors on all sides. For normal cells, this usually triggers contact inhibition, causing them to stop dividing and enter a quiescent phase.

**287.** (D) Cadherins
Contact inhibition is primarily mediated by cadherins, which are a type of cell-cell junctional protein. When cadherins on adjacent cells bind to each other, they initiate signaling pathways that communicate the high cell density to the nucleus. This feedback leads to the arrest of the cell cycle, ensuring that the population does not grow beyond a single, organized layer.

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**288.** (C) 70 - 85%
To preserve maximum viability, a culture should ideally be subcultured when it reaches 70 to 85% confluency. At this stage, the cells are still in the exponential growth phase and have not yet been affected by the nutrient depletion or toxic buildup that occurs at full confluency. Subculturing now ensures that the cells are healthy and recover quickly.

**289.** (C) Resemble epithelial cells (cuboidal/ polygonal) but their exact origin is unconfirmed
The term epithelioid describes cultured cells that resemble epithelial cells, appearing cuboidal or polygonal, but their exact biological origin is unconfirmed. These cells grow in tight patches or sheets, similar to a true epithelium. This descriptor is used when the morphological characteristics suggest an epithelial nature, but definitive markers to prove the specific tissue of origin are not available.

**290.** (D) I, II and III
Cultured cells show distinct morphologies: fibroblastic cells are bipolar and elongated, epithelial-like cells are polygonal and grow in patches, and lymphoblast-like cells are spherical and grow in suspension. All these descriptions accurately reflect the characteristic appearances of these cell types. Identifying these shapes is a fundamental skill for researchers to monitor the health and identity of their cell cultures.

**291.** (A) Membrane blebbing
Membrane blebbing is a classic indicator of early apoptosis in cultured cells. During this programmed cell death process, the cytoskeleton is reorganized, causing the cell membrane to form irregular bulges or blebs. This is a controlled and orderly event, distinct from the swelling and rupture seen in necrosis. Recognizing these morphological changes allows researchers to identify the pathways leading to death.

**292.** (C) Cellular swelling and random membrane rupture
Necrosis in cell culture is typically characterized by cellular swelling and random membrane rupture. Unlike the programmed and tidy process of apoptosis, necrosis is a passive and disorganized form of cell death caused by severe injury or environmental stress. As the membrane fails, the cell's contents spill into the surrounding medium, often affecting the health of neighboring cells in culture.

**293.** (B) Mutagenic potential of a compound
The Ames test on cultured cells evaluates the mutagenic potential of a compound. It is a biological assay designed to determine if a chemical can cause mutations in DNA. While traditionally performed using bacteria, modified versions using mammalian cells provide insights into how substances might affect human health. This test is a critical step in the safety evaluation of drugs.

**294.** (D) The extraction and purification of the desired product (e.g., antibody)
Downstream processing refers to the extraction and purification of the desired product from the cell culture or fermentation broth. After the cells have produced the protein or antibody in the bioreactor, the mixture must be processed to remove cells, debris, and media components. This stage is critical for ensuring the purity, safety, and effectiveness of biological therapeutics for use.

NODIA

**295.** (A) Protein A affinity chromatography
Protein A affinity chromatography is generally the first step in the downstream processing of monoclonal antibodies. Because of its highly specific binding to the Fc region of IgG, it allows for the capture of the antibody directly from the complex culture supernatant. This single step provides high purity and concentration, significantly simplifying the subsequent purification stages and overall processing.

**296.** (C) Chemically defined, animal-component-free media
A major ethical and scientific push in modern cell culture is the replacement of FBS with chemically defined, animal-component-free media. This shift addresses concerns about animal welfare and the high variability and risk of contamination associated with using serum. These synthetic media provide a more controlled and reproducible environment, which is essential for the standardized production of biological drugs.

**297.** (A) In glass
The term in vitro literally translates to in glass. It refers to biological processes or experiments conducted in a controlled environment outside of

a living organism, such as in test tubes, flasks, or petri dishes. This approach allows researchers to study cells and tissues in isolation, providing a powerful way to understand fundamental biology and test the effects of treatments.

**298.** (D) Ribosomes
Ribosomes are strictly essential for in vitro translation systems derived from cells. These molecular machines are responsible for reading messenger RNA and synthesizing proteins by linking amino acids together. In a cell-free system, all the necessary components for translation, including ribosomes and enzymes, are extracted from the cell, allowing researchers to study protein synthesis in a simplified and controlled environment.

**299.** (D) 96, 384, or 1536-well microtiter plates
High-throughput screening using cultured cells relies heavily on multi-well microtiter plates, such as those with 96, 384, or 1536 wells. These plates allow for the simultaneous testing of thousands of different compounds or conditions in a small format. Combined with automated handling and detection systems, HTS is a cornerstone of modern drug discovery and large-scale biological research in laboratories.

**300.** (A) The volumetric titer (mg/L) of the secreted monoclonal antibody
The ultimate goal of optimizing a fed-batch bioreactor for hybridomas is to maximize the volumetric titer of the secreted monoclonal antibody. By carefully controlling nutrient addition and environmental parameters, researchers aim to keep cells productive for as long as possible. A higher titer means more antibody is produced per liter, significantly improving the efficiency and economics of large-scale manufacturing.

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# CHAPTER 06

# Reproductive Biotechnology and Related Techniques

2021

**1.** Which hormone is primarily responsible for the recruitment of the follicular wave in livestock?
(A) Follicle-stimulating hormone
(B) Luteinizing pituitary hormone
(C) Progesterone steroid hormone
(D) Prostaglandin $F_2$-alpha hormone

2021

**2.** Identify the INCORRECT statement regarding the corpus luteum in bovines.
(A) It secretes progesterone to maintain pregnancy
(B) It is lysed by endogenous prostaglandin $F_2$ alpha
(C) It secretes high levels of estrogen
(D) It forms from the remnants of the ruptured follicle

2005

**3.** What is the correct sequence of events during natural fertilization in mammals?
(A) Cortical reaction, acrosome reaction, capacitation
(B) Capacitation, acrosome reaction, cortical reaction
(C) Acrosome reaction, capacitation, cortical reaction
(D) Capacitation, cortical reaction, acrosome reaction

2014

**4.** Match the events of mammalian embryo development (List I) with their defining features (List II).
List I: (Events of mammalian embryo development)
P. Cleavage
Q. Compaction
R. Blastulation
S. Hatching
List II: (Defining features)
1. Escape from the zona pellucida
2. Formation of a fluid-filled cavity
3. Blastomeres maximize cell-to-cell contact
4. Mitotic divisions without cytoplasmic growth
(A) P-4, Q-3, R-2, S-1
(B) P-3, Q-2, R-1, S-4
(C) P-2, Q-1, R-4, S-3
(D) P-1, Q-4, R-3, S-2

2017

**5.** Which of the following best defines sperm capacitation?
(A) The physical penetration and binding of the zona pellucida
(B) Sperm maturation within the female reproductive tract
(C) The exocytosis of hydrolytic enzymes from the sperm head
(D) The nuclear fusion of male and female pronuclei

NODIA

2011

**6.** What is the most appropriate pharmacological protocol for estrus synchronization in cyclic cows?
(A) A single injection of human chorionic gonadotropin to induce ovulation
(B) Daily systemic administration of oxytocin during the luteal phase
(C) Progestin device followed by prostaglandin $F_2$ alpha
(D) Continuous daily administration of follicle-stimulating hormone

2018

**7.** Consider the following statements about gonadotropins in superovulation:
I. eCG has a longer halF-life than FSH in cattle.
II. FSH must be administered in multiple doses due to its short halF-life.
III. hCG is primarily used to induce luteolysis.
Which of the above statements are correct?
(A) I and II
(B) I and III
(C) II and III

halt the cell cycle. The loss of this property is a hallmark of cancerous transformation.

**259.** (D) Extra-nuclear fluorescent particulate matter in the cytoplasm
A classic sign of Mycoplasma contamination is the presence of extra-nuclear fluorescent particulate matter in the cytoplasm when using DAPI or Hoechst stain. Because Mycoplasma are tiny DNA-containing organisms that adhere to the outside of the host cell, they appear as small dots scattered around the large host nucleus. This staining pattern is a clear visual indicator of contamination.

**260.** (A) I, II and IV
Trypan blue stains dead cells, while MTT measures metabolic activity as an indicator of viability. LDH release is a reliable marker for membrane damage and cytotoxicity. Together, these assays provide a comprehensive view of cell health. However, propidium iodide only stains dead cells, and Calcein-AM specifically stains live cells. Thus, statements I, II, and IV are correct.

**261.** (B) Serum
Cell synchronization can be achieved by removing serum from the culture medium. This process, known as serum starvation, deprives cells of the growth factors and hormones needed to progress through the cell cycle. As a result, most cells will arrest in the $G_0$ or $G_1$ phase. When serum is later added back, the cells will begin dividing simultaneously.

**262.** (D) Double thymidine block
The double thymidine block is a chemical method that induces cell synchronization by reversibly arresting cells in the S phase. Excess thymidine inhibits DNA synthesis through a feedback mechanism involving nucleotide pools. By applying the block, washing the cells, and then applying it again, researchers can ensure that a high percentage of the cells are at the same point.

**263.** (A) High-efficiency particulate air (HEPA) filtration of the air supply
A laminar flow hood provides a sterile environment through HEPA filtration of the air supply. The filter removes 99.97% of particles that are 0.3 micrometers or larger, including bacteria and fungal spores. The hood creates a steady, unidirectional flow of clean air that prevents contaminated room air from entering the work area, ensuring that cell culture procedures are clean.

**264.** (C) Class II
A Class II biological safety cabinet is the standard equipment for routine, non-hazardous animal cell culture. It provides protection for the product, the environment, and the operator. By using HEPA-filtered air and a specific airflow pattern, these cabinets maintain a sterile work area while preventing the escape of any biological materials, making them essential for safe laboratory work.

**265.** (C) 70%
In a Class II Type $A_2$ biological safety cabinet, approximately 70% of the air is typically recirculated within the cabinet. This air is passed through a HEPA filter to maintain the sterile environment in the work zone. The remaining 30% of the air is exhausted through another HEPA filter, ensuring that any potential contaminants are removed before release.

**266.** (D) Glove box
A Class III biological safety cabinet is often referred to as a glove box. This type of cabinet provides the highest level of protection by being completely gas-tight and separated from the operator by physical barriers. Researchers work through heavy-duty gloves attached to the front of the unit. These are used for handling highly hazardous pathogens that require containment.

**267.** (B) The number of times the culture has been subcultured (split)
The passage number of a cell line refers to the number of times the culture has been subcultured or split. Each time cells reach confluency and are transferred to new vessels, the passage number increases by one. Tracking this number is important because prolonged culture can lead to genetic changes, so researchers often limit the passages used for experiments.

**268.** (C) Phenotypic and genetic drift
High passage numbers in continuous cell lines are associated with phenotypic and genetic drift. Over time, the constant division and selection pressures of the laboratory environment can lead to mutations and changes in cell behavior. The cells may lose specific characteristics or show altered responses to drugs. To ensure

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2005

**18.** What is the fundamental biological challenge in generating monozygotic twins via embryo bisection?
(A) Complete loss of the zona pellucida prevents any further development
(B) Bisection automatically alters the genetic sex of one half
(C) Reduction in cell number may compromise halF-embryo viability
(D) Bisected embryos cannot be cryopreserved

2014

**19.** Arrange the following steps of Somatic Cell Nuclear Transfer (SCNT) in the correct operational sequence:
I. Electrofusion
II. Donor cell synchronization
III. Oocyte enucleation
IV. Oocyte activation
(A) II, III, I, IV
(B) III, II, IV, I
(C) II, I, III, IV
(D) III, IV, I, II

2015

**20.** Which phenomenon is commonly associated with epigenetic reprogramming failures in cloned livestock?
(A) Polyspermy
(B) Freemartinism
(C) Robertsonian translocation
(D) Large Offspring Syndrome

2021

**21.** What is the primary advantage of using CRISPR-Cas9 over traditional homologous recombination for genome editing in livestock?
(A) It requires absolutely no custom synthetic guide RNA
(B) Higher targeted double-strand break efficiency
(C) It relies entirely on random retroviral host integration
(D) It completely avoids any offF-target genomic cleavage events

2009

**22.** Consider the following statements regarding gene pharming:
I. Expression vectors use mammary-gland specific promoters.
II. Casein promoters ensure the transgene product is secreted in milk.
III. Bioreactor animals produce recombinant proteins in their blood only.
IV. Beta-lactoglobulin is a commonly used promoter.
V. It aims to mass-produce valuable human therapeutic proteins.
Which of the above statements are incorrect?
(A) I and II
(B) III only
(C) IV and V
(D) I, II and V

NODIA

2019

**23.** Match the stem cell types (List I) with their specific developmental potential (List II).
List I: (Stem cell types)
P. Totipotent
Q. Pluripotent
R. Multipotent
S. Unipotent
List II: (Developmental potential)
1. Can form all embryonic and extraembryonic tissues
2. Can form all three germ layers but not the placenta
3. Can form multiple cell types within a specific lineage
4. Can form only one specific cell type
(A) P-2, Q-1, R-3, S-4
(B) P-1, Q-2, R-3, S-4
(C) P-1, Q-3, R-2, S-4
(D) P-2, Q-3, R-1, S-4

2022

**24.** Which marker is NOT classically used for the characterization of embryonic stem cells?
(A) CD45
(B) Oct-4
(C) Nanog
(D) SSEA

2010

**25.** What is the main clinical application of induced pluripotent stem cells (iPSCs) in veterinary medicine?
(A) Generating nutritional feed additives for livestock
(B) Generating patient-specific cells for therapy
(C) Enhancing efficiency of traditional artificial insemination
(D) Inducing multiple superovulations in elite dairy cattle

2004

**26.** In the endocrine regulation of the estrous cycle, which hormone triggers the preovulatory LH surge?

(A) Progesterone
(B) Estrogen
(C) Inhibin
(D) Prolactin

2023
**27.** Identify the correct statement regarding the acrosome reaction.
(A) It is an irreversible exocytotic process required for zona pellucida penetration
(B) It occurs spontaneously and immediately upon ejaculation
(C) It prevents sperm from binding to any zona receptors
(D) It represents an entirely reversible physiological process

2012
**28.** Which event marks the completion of the cleavage stage in pre-implantation embryo development?
(A) Extrusion of the first polar body
(B) Hatching from the zona pellucida
(C) Formation of the morula
(D) Implantation into the endometrium

2018
**29.** Analyze the given assertion and reason, then select the correct option:
**Assertion (A):** $PGF_2$ alpha is administered to regress the existing corpus luteum.
**Reason (R):** Regression of the corpus luteum causes a drop in progesterone, allowing estrus to occur.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2001
**30.** Which pharmacological protocol relies on a CIDR device?
(A) Induction of superovulation
(B) In vitro capacitation
(C) Estrus synchronization
(D) Polyspermy block

2015
**31.** What defines an excellent (Grade 1) embryo according to IETS guidelines?
(A) Moderate irregularities in blastomere size with 20% cellular fragmentation
(B) Symmetrical, spherical mass with minimal extruded cells
(C) Major structural irregularities with at least 50% cellular fragmentation
(D) Dead, dark, or degenerating blastomeres within the zona pellucida

2013
**32.** Select the correct sequence of media types used in the step-wise culture of mammalian embryos.
(A) Cleavage medium followed by blastocyst medium
(B) Blastocyst medium followed by maturation medium
(C) Capacitation medium followed by cleavage medium
(D) Maturation medium followed by capacitation medium

2001
**33.** Consider the following statements about somatic cell co-culture systems:
I. They were historically used to overcome the in vitro developmental block.
II. They secrete embryotrophic factors.
III. They have been entirely replaced by serum-containing media.
Which of the above statements are correct?
(A) I and II
(B) I and III
(C) II and III
(D) I, II and III

2005
**34.** Which technique involves the physical injection of a single spermatozoon directly into the ooplasm?
(A) SUZI
(B) PZD
(C) ICSI
(D) MOET

2021
**35.** What is the primary difference in DNA content between bovine X and Y chromosome-bearing sperm?
(A) The X sperm contains approximately 3.8% more DNA
(B) The Y sperm contains approximately 3.8% more DNA
(C) The X sperm contains 10% less DNA
(D) There is no measurable difference in DNA content

2021
**36.** Which method of embryo sexing targets specific alleles but does not require live cells for continuous culture?
(A) Karyotyping of dividing cells

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(B) H-Y antigen immunological assay
(C) Flow cytometry sorting
(D) PCR-based amplification

2019

**37.** Match the cryoprotectant properties (List I) with their descriptions (List II).
List I: (Cryoprotectant properties)
P. Permeating CPA
Q. Non-permeating CPA
R. Toxicity
S. Vitrification
List II: (Descriptions)
1. Ice-free solidification
2. Enters the cell to prevent intracellular ice
3. Remains extracellular to dehydrate the cell
4. Increases with higher CPA concentration and temperature
(A) P-1, Q-2, R-3, S-4
(B) P-2, Q-3, R-4, S-1
(C) P-3, Q-4, R-1, S-2
(D) P-4, Q-1, R-2, S-3

2024

**38.** Identify the INCORRECT statement concerning vitrification.
(A) It requires highly elevated, toxic concentrations of cryoprotectants
(B) It involves extremely high, ultra-rapid physical cooling rates
(C) It relies on extracellular ice to dehydrate cells
(D) It completely avoids the formation of any damaging ice crystals

2001

**39.** What tool is essential for the mechanical bisection of embryos?
(A) High-speed flow cytometer
(B) Real-time PCR thermocycler
(C) High-voltage electroporator
(D) Micromanipulator with blade

2015

**40.** Which factor is fundamentally responsible for Large Offspring Syndrome in cloned ruminants?
(A) Abnormal epigenetic modification of genes
(B) Excessively high expression of the male SRY gene
(C) Pathogenic bacterial infection of the surrogate mother
(D) Incorrect use of non-permeating cryoprotectants

2001

**41.** Analyze the given assertion and reason, then select the correct option:
**Assertion (A):** The donor somatic cell is usually synchronized in the $G_0/G_1$ phase of the cell cycle.
**Reason (R):** $G_0/G_1$ synchronization facilitates better genomic reprogramming by the recipient oocyte cytoplasm.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2013

**42.** Which transgenic animal production method utilizes the natural ability of a virus to integrate its genome into the host DNA?
(A) Pronuclear microinjection
(B) Retroviral vectors
(C) Sperm-mediated gene transfer
(D) Somatic cell nuclear transfer

2011

**43.** Consider the following statements about gene editing tools:
I. ZFNs utilize zinc finger DNA-binding domains.
II. TALENs rely on transcription activator-like effectors.
III. CRISPR-Cas9 uses a guide RNA to target specific DNA sequences.
IV. Homologous recombination is generally more efficient than CRISPR-Cas9.
Which of the above statements is/are incorrect?
(A) I and II
(B) III only
(C) IV only
(D) I, II and III

2017

**44.** What is the defining characteristic of a multipotent stem cell?
(A) It retains the high capacity to develop into a complete living organism
(B) It can differentiate into specialized cells from all three germ layers
(C) It forms a limited number of cell types in a lineage
(D) It completely lacks any ability to divide or selF-renew in vitro

2024

**45.** Which of the following best describes the stem cell niche?

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(D) I, III and IV

2001

**83.** Which pharmacological agent is a synthetic analogue of $PGF_2$ alpha used in estrus synchronization?
(A) Cloprostenol
(B) Norgestomet
(C) Buserelin
(D) Melengestrol acetate

2024

**84.** What happens during the compaction stage of mammalian development?
(A) The embryo physically shrinks in its overall total outer diameter
(B) The outer zona pellucida becomes highly permeable to swimming sperm
(C) The active embryonic genome is permanently and completely silenced
(D) Blastomeres flatten to maximize contacts

2015

**85.** Match the genome editing tools (List I) with their DNA recognition mechanism (List II).
List I: (Genome editing tools)
P. Zinc Finger Nucleases (ZFNs)
Q. TALENs
R. CRISPR-Cas9
S. Homologous Recombination
List II: (DNA recognition mechanism)
1. Protein domain binding to 3-4 base pairs
2. Single guide RNA
3. Extensive sequence homology
4. Protein domain binding to a single nucleotide
(A) P-1, Q-4, R-2, S-3
(B) P-4, Q-1, R-3, S-2
(C) P-2, Q-3, R-1, S-4
(D) P-3, Q-2, R-4, S-1

2018

**86.** Which application is an example of gene pharming?
(A) Creating a transgenic pig that digests plant phosphorus more efficiently
(B) Knocking out the endogenous myostatin gene to increase beef cattle muscle mass
(C) Producing therapeutic protein in goat milk
(D) Developing a genetically modified sheep with highly refined fleece wool

2015

**87.** What is the primary cause of epigenetic reprogramming failures in cloned embryos?
(A) Incorrect use of standard culture media instead of sequential media systems
(B) Accidental physical damage sustained by the oocyte during micro-enucleation
(C) Subclinical pathogenic viral or bacterial infection of the surrogate mother
(D) Incomplete donor nucleus chromatin remodeling

2024

**88.** Identify the INCORRECT statement about Partial Zona Dissection (PZD).
(A) It guarantees monospermic fertilization
(B) It involves making a slit in the zona pellucida
(C) It facilitates sperm entry into the perivitelline space
(D) It is an early form of micro-assisted fertilization

2002

**89.** Which concept explains how different populations of stem cells are maintained in the body?
(A) The Hayflick limit
(B) The stem cell niche hypothesis
(C) The endosymbiotic theory
(D) The central dogma of molecular biology

NODIA

2007

**90.** Analyze the given assertion and reason, then select the correct option:
**Assertion (A):** Flow cytometry sorts sperm into X and Y populations based on fluorescence intensity.
**Reason (R):** X-chromosome bearing sperm absorb more fluorescent DNA dye because they have a larger DNA mass than Y-bearing sperm.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2015

**91.** What is the typical required length of donor-recipient estrus synchrony for successful bovine embryo transfer?
(A) Plus or minus 24 hours
(B) Exactly the same hour
(C) Plus or minus 5 days
(D) Synchrony is not required for blastocyst transfer

2014

**92.** Consider the following statements about IVC of embryos:
I. Defined media completely lack any protein supplementation.

2009

**54.** What is a significant limitation of using retroviral vectors for producing transgenic livestock?
(A) They are entirely unable to integrate into host genome sequences
(B) They exclusively target and modify host mitochondrial DNA
(C) Limited gene packaging capacity
(D) They require electrical pulse-mediated membrane fusion

2018

**55.** Analyze the given assertion and reason, then select the correct option:
**Assertion (A):** Homologous recombination allows for the targeted disruption of a specific gene.
**Reason (R):** It utilizes a targeting vector containing sequences homologous to the target locus to facilitate crossing over.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2023

**56.** Which of the following best defines gene pharming?
(A) Using transgenic animals to produce drugs
(B) Producing high-yield genetically modified crops for feed
(C) Knocking out specific disease-associated genes in livestock
(D) Using exogenous reproductive drugs to synchronize estrus cycles

2005

**57.** Which promoter is most appropriately used to direct transgene expression specifically to the milk of a bioreactor animal?
(A) Cytomegalovirus (CMV) promoter
(B) Beta-lactoglobulin promoter
(C) Beta-actin promoter
(D) Ubiquitin promoter

2022

**58.** Identify the INCORRECT statement regarding the integration of economically important genes in livestock.
(A) It can enhance wool growth in sheep
(B) It always results in severe physiological abnormalities
(C) It can confer resistance to specific diseases, like mastitis
(D) It aims to improve overall production traits

2015

**59.** What role does the transcription factor Nanog play in embryonic stem cells?
(A) It maintains the state of pluripotency
(B) It promotes differentiation into the mesoderm
(C) It initiates cellular apoptosis
(D) It suppresses telomerase activity

2005

**60.** How are induced pluripotent stem cells (iPSCs) generated?
(A) By harvesting pluripotent cells from the inner cell mass of a blastocyst
(B) By directly fusing a viable embryonic stem cell with a tissue macrophage
(C) By reprogramming somatic cells with transcription factors
(D) By isolating and expanding multipotent progenitor cells from bone marrow

2013

**61.** Which hormone is primarily responsible for the maintenance of the corpus luteum during early pregnancy in cattle?
(A) Prostaglandin $F_2$ alpha
(B) Follicle-stimulating hormone
(C) Oxytocin
(D) Interferon-tau

2019

**62.** Consider the following statements about sperm capacitation:
I. It involves the removal of seminal plasma proteins from the sperm surface.
II. It increases intracellular calcium levels.
III. It results in hyperactivated motility.
IV. It takes place entirely in the epididymis.
V. It prepares the sperm for the acrosome reaction.
Which of the above statements are correct?
(A) I, II, III and V
(B) I, II, IV and V
(C) II, III, IV and V
(D) I, III, IV and V

2003

**63.** The developmental block observed in early in vitro mammalian embryo culture typically coincides with which major biological transition?
(A) The transition from maternal to embryonic genome activation
(B) The transition from the morula to the blastocyst stage
(C) The transition from aerobic to anaerobic glycolysis
(D) The transition from fertilization to the first

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(B) To act as active physiological surrogate hosts for young embryos
(C) To scavenge radicals and secrete growth factors
(D) To trigger or initiate the physiological acrosome reaction

2023

**119.** What technology allows the generation of embryonic stem cells strictly matching the genetic profile of an adult patient?
(A) Standard physical pronuclear microinjection of DNA
(B) Intracytoplasmic sperm injection of single gametes
(C) High-speed flow cytometry cell sorting and analysis
(D) SCNT followed by blastocyst isolation

2014

**120.** Analyze the given assertion and reason, then select the correct option:
**Assertion (A):** Rapid thawing is generally required for embryos frozen via slow-freezing protocols.
**Reason (R):** Rapid thawing prevents the recrystallization of small ice crystals into large, damaging ones.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2021

**121.** What is the major application of subzonal insemination (SUZI)?
(A) Sorting sperm cells prior to flow cytometry analysis
(B) Aiding fertilization through a hardened zona
(C) Inducing superovulatory responses in poor cow responders
(D) Extracting the first polar body for chromosomal analysis

2004

**122.** Consider the following statements about SSEA (Stage-Specific Embryonic Antigens):
I. They are cell surface molecules.
II. They are used as markers to identify embryonic stem cells.
III. Their expression patterns change as the stem cell differentiates.
IV. They are uniquely found in the mitochondria.
Which of the above statements are correct?
(A) I, II and IV
(B) I, II and III
(C) II, III and IV
(D) I, III and IV

2025

**123.** What biological parameter dictates the timing of embryo flushing in a superovulated cow?
(A) Embryo transit time from oviduct to uterus
(B) The total time required for the outer zona pellucida to dissolve
(C) The average physiological speed of the sperm acrosome reaction
(D) The biological halF-life profile of the administered FSH hormone

2012

**124.** What defines a 'knockout' animal?
(A) An animal harboring extra transgenic copies of an inserted gene
(B) An animal developed and gestated completely without a surrogate mother
(C) An animal with a disrupted or inactivated gene
(D) An animal engineered to express a highly fluorescent reporter gene

NODIA

2011

**125.** Arrange the following components of a gene pharming construct in the correct logical sequence from 5' to 3':
I. Gene of interest (e.g., human antithrombin)
II. Mammary-specific promoter
III. Polyadenylation signal
(A) I, II, III
(B) II, I, III
(C) III, I, II
(D) II, III, I

2002

**126.** Which feature is most characteristic of apoptosis in an embryo?
(A) Acute swelling and subsequent rupture of the outer cell membrane
(B) Immediate massive necrosis occurring across the inner cell mass
(C) Fragmentation and chromatin condensation
(D) Rapid, highly uncontrolled mitotic cellular proliferation events

2022

**127.** Identify the INCORRECT pair regarding gamete biology.
(A) Syngamy - Fusion of male and female pronuclei

III. They are non-invasive compared to PCR.
Which of the above statements is/are INCORRECT?
(A) I and II
(B) II only
(C) I and III
(D) III only

2019
**74.** Which of the following is a primary advantage of vitrification over slow-freezing?
(A) It uses much lower and safer concentrations of toxic cryoprotectants
(B) It eliminates programmable freezers
(C) It routinely allows for the successful freezing of whole adult tissues
(D) It completely prevents the long-term need for liquid nitrogen

2004
**75.** In follicular wave dynamics, what characterizes the 'dominant' follicle?
(A) It secretes inhibin to suppress FSH
(B) It rapidly undergoes cellular atresia early in the cycle
(C) It requires abnormally high levels of progesterone to ovulate
(D) It completely lacks active luteinizing hormone receptors

2009
**76.** What is the role of cortical granules during fertilization?
(A) They chemically attract multiple sperm to the outer oocyte
(B) They provide metabolic energy for the first embryo cleavage
(C) They release enzymes to harden the zona
(D) They temporarily prevent the normal completion of meiosis II

2009
**77.** Which of the following enzymes is critical for the targeted gene disruption achieved by CRISPR?
(A) DNA ligase
(B) Reverse transcriptase
(C) Taq polymerase
(D) Cas9 endonuclease

2025
**78.** Which of the following is an INCORRECT pairing of a stem cell type and its primary source?
(A) Embryonic stem cells (ESCs) - Trophoblast of the blastocyst
(B) Induced pluripotent stem cells (iPSCs) - Reprogrammed adult somatic cells
(C) Mesenchymal stem cells (MSCs) - Bone marrow or adipose tissue
(D) Hematopoietic stem cells (HSCs) - Bone marrow

2016
**79.** What distinguishes multipotent adult stem cells from embryonic stem cells?
(A) Adult stem cells have a much higher clinical risk of forming teratomas
(B) Adult stem cells are restricted to certain lineages
(C) Adult stem cells can spontaneously generate a complete living organism
(D) Adult stem cells do not naturally exist in any mammalian species

2010
**80.** Analyze the given assertion and reason, then select the correct option:
**Assertion (A):** Foley catheters are commonly used for non-surgical embryo flushing in cattle.
**Reason (R):** The inflatable balloon of the Foley catheter seals the uterine horn to prevent the loss of flushing fluid.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2005
**81.** Which is the LEAST relevant factor when evaluating an oocyte for IVM?
(A) Sex chromosome of the oocyte
(B) Uniform homogeneity of the ooplasm
(C) Total number of cumulus cell layers
(D) Presence of a fully formed outer zona pellucida

2018
**82.** Consider the following statements regarding embryo splitting:
I. It effectively doubles the number of viable embryos from a single donor.
II. It requires a micromanipulator.
III. It results in dizygotic twins.
IV. Pregnancy rates per halF-embryo are usually slightly lower than whole embryos.
Which of the above statements are correct?
(A) I, II and IV
(B) I, II and III
(C) II, III and IV

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(A) The specific intracellular organelles found within a stem cell
(B) The synthetic culture medium used for in vitro oocyte maturation
(C) The complex genetic construct used to artificially induce pluripotency
(D) The microenvironment regulating stem cell behavior

2025
**46.** What is the effect of administering a luteolytic agent during the luteal phase of the estrous cycle?
(A) Sudden induction of immediate ovulation
(B) Long-term maintenance of active pregnancy
(C) Premature luteal regression
(D) Spontaneous development of large follicular cysts

2024
**47.** Which receptor on the zona pellucida is primarily responsible for initial sperm binding in mammals?
(A) ZP1
(B) ZP3
(C) ZP2
(D) ZP4

2016
**48.** Identify the correct pairing of an embryo developmental stage with its biological process.
(A) Cleavage - Massive increase in total embryonic volume
(B) Blastulation - Direct rupture of the outer zona pellucida
(C) Hatching - Separation of the inner cell mass and trophoblast
(D) Compaction - Tight junction formation

2022
**49.** Arrange the following steps of superovulation and embryo transfer in the correct chronological sequence:
I. Embryo flushing
II. Prostaglandin administration to donor
III. Artificial insemination
IV. FSH administration to donor
(A) IV, II, III, I
(B) II, IV, I, III
(C) IV, III, II, I
(D) II, I, IV, III

2007
**50.** What is the primary biological reason for washing embryos prior to transfer or freezing?
(A) To physically trigger the natural blastocyst hatching process
(B) To successfully initiate in vitro sperm head capacitation
(C) To remove infectious agents on the zona pellucida
(D) To completely dissolve intracellular lipid droplets in blastomeres

NODIA

2001
**51.** Consider the following statements regarding Cumulus-Oocyte Complex (COC) grading:
I. Grade A COCs have a compact, multi-layered cumulus investment.
II. Grade B COCs have completely expanded and dark cumulus layers.
III. Denuded oocytes lack a cumulus cell layer entirely.
IV. Grade A COCs are generally preferred for IVM.
Which of the above statements are correct?
(A) I, II and IV
(B) I, III and IV
(C) II, III and IV
(D) I, II and III

2018
**52.** Which mechanism acts as the primary slow block to polyspermy in mammalian oocytes?
(A) Electrical depolarization of the oocyte plasma membrane
(B) Spontaneous enzymatic degeneration of the sperm tail
(C) Significant physical shrinkage of the perivitelline space
(D) Cortical granule release altering the zona

2023
**53.** Match the assisted reproductive technologies (List I) with their primary application (List II).
List I: (Assisted reproductive technologies)
P. IVF
Q. ICSI
R. SCNT
S. Flow cytometry
List II: (Primary application)
1. Treating severe male factor infertility
2. In vitro generation of embryos from collected gametes
3. Producing somatic cell clones
4. Sorting sperm by sex chromosome
(A) P-2, Q-1, R-3, S-4
(B) P-1, Q-2, R-4, S-3
(C) P-3, Q-4, R-2, S-1
(D) P-4, Q-3, R-1, S-2

II. Bovine serum albumin (BSA) is often used in semi-defined media.
III. Amino acids are generally omitted from modern sequential media.
IV. Low oxygen tension (5%) improves in vitro embryo development.

Which of the above statements are correct?
(A) I and II
(B) II and IV
(C) I, II and IV
(D) II, III and IV

2006
**93.** What is the fundamental biological challenge in generating monozygotic twins via embryo bisection?
(A) Direct microinjection of plasmid DNA into the sperm head before IVF
(B) Using a modified retroviral vector to infect testicular germ cells
(C) Completely replacing the sperm nucleus with a donor somatic nucleus
(D) Sperm binding exogenous DNA and carrying it to the egg

2011
**94.** Which hormone profile is characteristic of the luteal phase of the estrous cycle?
(A) High estrogen, low progesterone
(B) High FSH, high oxytocin
(C) High progesterone, low estrogen
(D) Low progesterone, high LH

2014
**95.** What structural change marks the transformation of a morula into a blastocyst?
(A) Meiotic extrusion of a polar body
(B) Blastocoele cavity formation
(C) Complete shedding of the zona pellucida
(D) Rapid, immediate attachment to the endometrium

2001
**96.** Which is a recognized disadvantage of using eCG (PMSG) for superovulation instead of FSH?
(A) It requires highly frequent labor-intensive injections every twelve hours
(B) It is extremely cost-prohibitive and hard to obtain compared to FSH
(C) It causes immediate, premature regression of active corpora lutea
(D) Long halF-life causes cystic ovaries

2016
**97.** Identify the correct statement regarding embryo evaluation.
(A) An embryo with severe fragmentation is graded as poor or degenerate
(B) IETS Grade 4 embryos are highly suitable for freezing and export
(C) Embryo grading focuses solely on the genetic sex of the embryo
(D) The presence of a zona pellucida automatically grades an embryo as excellent

2014
**98.** What defines the process of oocyte enucleation in SCNT?
(A) The microinjection-mediated addition of a new somatic nucleus to the oocyte
(B) Artificial fertilization of the oocyte using non-capacitated sperm
(C) Removing the oocyte's chromosomes
(D) Non-sperm chemical activation of the recipient oocyte cytoplasm

2005
**99.** Which technique is most appropriate for evaluating the ploidy of a biopsied embryo?
(A) Karyotyping
(B) Flow cytometry
(C) ELISA for H-Y antigen
(D) Standard PCR

2010
**100.** Match the physiological processes (List I) with their corresponding locations in vivo (List II).

List I: (Physiological processes)
P. Sperm capacitation
Q. Fertilization
R. Cleavage divisions
S. Blastocyst hatching

List II: (Corresponding locations in vivo)
1. Uterus
2. Ampulla-isthmic junction
3. Female reproductive tract (cervix/uterus/oviduct)
4. Oviductal transit

(A) P-3, Q-2, R-4, S-1
(B) P-2, Q-3, R-1, S-4
(C) P-4, Q-1, R-3, S-2
(D) P-1, Q-4, R-2, S-3

2017
**101.** What is the biological function of the acrosome reaction?
(A) To effectively prevent polyspermic fertilization
(B) To penetrate the zona pellucida
(C) To supply essential metabolic nutrients to the oocyte

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(D) To physically eject the flagellum or sperm tail

2001

**102.** Analyze the given assertion and reason, then select the correct option:
**Assertion (A):** Pluripotent cells can form tumors called teratomas when injected into immune-deficient mice.
**Reason (R):** Pluripotent cells have the capacity to differentiate haphazardly into tissues representing all three germ layers in vivo.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2019

**103.** Which is a correct pair regarding a gene and its effect when integrated into livestock?
(A) Myostatin gene disruption - Significant decrease in total muscle mass
(B) Prion protein genetic overexpression - Full resistance to mad cow disease
(C) Mammary lysozyme gene knockout - Greatly enhanced dairy milk production
(D) Phytase transgene in pigs - Reduced phosphorus pollution

2009

**104.** Consider the following statements about follicular aspiration for IVM:
I. It can be performed on live animals using ultrasound guidance (OPU).
II. It requires the animal to be euthanized.
III. It harvests cumulus-oocyte complexes directly from antral follicles.
IV. It yields only mature, metaphase II oocytes.
Which of the above statements are correct?
(A) I and III
(B) I and IV
(C) II and III
(D) I, III and IV

2021

**105.** What is the role of a permeating cryoprotectant during slow freezing?
(A) To freeze extracellular free water as quickly as possible
(B) To replace cell water and prevent ice crystallization
(C) To selectively dissolve outer cell membrane components
(D) To biochemically generate metabolic ATP during storage

2018

**106.** Which parameter directly determines the sorting efficiency of a flow cytometer for semen sexing?
(A) The emission speed and wavelength of the laser
(B) Sperm head alignment during detection
(C) The physical length of the swimming sperm tail
(D) The progressive motility rate of the sperm cells

2013

**107.** Identify the INCORRECT statement regarding Large Offspring Syndrome (LOS).
(A) It is caused by viral infections during pregnancy
(B) It is defined by abnormally elevated and high birth weights
(C) It can lead to severe clinical dystocia and calving issues
(D) It is frequently found in somatic cell cloned calves and sheep

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2024

**108.** What is the primary function of TALENs in genome editing?
(A) To randomly integrate foreign transgenes into the host genome
(B) To serve as an efficient viral vector for therapeutic gene delivery
(C) To induce targeted genomic double-strand breaks
(D) To selectively block or inhibit ribosomal translation of mRNA

2019

**109.** Which sequence correctly orders the stages of the estrous cycle?
(A) Proestrus, Estrus, Metestrus, Diestrus
(B) Estrus, Proestrus, Diestrus, Metestrus
(C) Metestrus, Diestrus, Proestrus, Estrus
(D) Diestrus, Metestrus, Estrus, Proestrus

2017

**110.** What is the clinical significance of CD markers on adult stem cells?
(A) They trigger metabolic pathways causing immediate cellular apoptosis
(B) They are structural transmembrane proteins lacking diagnostic value
(C) They serve as surface markers for cell isolation
(D) They actively prevent the pluripotent stem

cell from differentiating

2011

**111.** Analyze the given assertion and reason, then select the correct option:
**Assertion (A):** The Ovsynch protocol includes the administration of both GnRH and prostaglandin $F_2$-alpha.
**Reason (R):** Prostaglandin $F_2$-alpha alone can effectively synchronize ovulation in anestrus cows.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2016

**112.** Which statement best describes the hatching mechanism of a blastocyst?
(A) The physical tearing of the zona pellucida by flagellar motion
(B) Complete dissolving of the outer zona pellucida by uterine acid
(C) The sudden, spontaneous disintegration of outer trophoblast cells
(D) Enzymatic degradation paired with blastocyst expansion

2010

**113.** Consider the following statements regarding gamete co-incubation during IVF:
I. Sperm concentration must be carefully optimized to prevent polyspermy.
II. Co-incubation typically lasts for 24 to 48 hours.
III. Capacitation agents are often present in the co-incubation medium.
IV. COCs are usually completely stripped of cumulus cells before co-incubation.
Which of the above statements are INCORRECT?
(A) I and III
(B) II and IV
(C) III and IV
(D) I, II and III

2001

**114.** Which vector is traditionally used to generate transgenic livestock via pronuclear microinjection?
(A) An engineered replication-deficient retroviral plasmid
(B) A live, replication-competent human adenovirus vector
(C) A linear DNA construct with gene and promoter
(D) An engineered sequence-specific zinc finger nuclease

2003

**115.** What is the fundamental basis of epigenetic reprogramming?
(A) Changing gene expression without altering DNA sequence
(B) Permanently altering the underlying nucleosome genomic DNA sequence
(C) Physically extracting entire chromosomes from the donor cell nucleus
(D) Artificially inducing genomic crossing over during mitotic division

2009

**116.** Match the hormones (List I) with their effect on the reproductive system (List II).
List I: (Hormones)
P. GnRH
Q. Progesterone
R. Oxytocin
S. Inhibin
List II: (Effect on the reproductive system)
1. Suppresses FSH release
2. Induces milk let-down and uterine contractions
3. Stimulates release of FSH and LH
4. Maintains pregnancy and suppresses estrus
(A) P-3, Q-4, R-2, S-1
(B) P-4, Q-3, R-1, S-2
(C) P-3, Q-1, R-4, S-2
(D) P-1, Q-4, R-2, S-3

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2013

**117.** Identify the correct statement regarding embryo transfer methodology.
(A) Embryos are always transferred to the uterine horn contralateral to the corpus luteum
(B) Embryos are deposited into the uterine horn ipsilateral to the corpus luteum
(C) Surgical embryo transfer is generally preferred over non-surgical due to lower costs
(D) The cervix of a cow in diestrus is easily penetrated without any manipulation

2025

**118.** What is the primary role of somatic cell co-culture in traditional embryo culture systems?
(A) To provide a completely sterile, chemically defined environment

cleavage division

2018

**64.** Which of the following is considered a non-permeating cryoprotectant?
(A) Ethylene glycol
(B) Dimethyl sulfoxide (DMSO)
(C) Propylene glycol
(D) Polyvinylpyrrolidone

2005

**65.** Identify the correct sequence of events in the generation of a transgenic animal via pronuclear microinjection.
(A) Zygote collection, DNA injection into pronucleus, embryo transfer, screening of offspring
(B) Embryo transfer, DNA injection into pronucleus, zygote collection, screening of offspring
(C) Zygote collection, embryo transfer, DNA injection into pronucleus, screening of offspring
(D) DNA injection into pronucleus, zygote collection, screening of offspring, embryo transfer

2015

**66.** Analyze the given assertion and reason, then select the correct option:
**Assertion (A):** Artificial activation of the reconstructed oocyte is necessary in SCNT.
**Reason (R):** The enucleation and fusion processes bypass the normal sperm-induced calcium oscillations.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2008

**67.** Which phenomenon refers to the shortened lifespan of clones due to shortened chromosomal ends?
(A) Telomere shortening
(B) Random X-chromosome inactivation
(C) Epigenetic genomic imprinting
(D) Severe mitochondrial heteroplasmy

2007

**68.** What is the main structural component targeted by Subzonal Insemination (SUZI)?
(A) The central ooplasm
(B) The germinal vesicle
(C) The first polar body
(D) Perivitelline space

2010

**69.** Match the superovulation hormones (List I) with their primary origin (List II).
List I: (Superovulation hormones)
P. eCG
Q. hCG
R. FSH
S. $PGF_2$ alpha
List II: (Primary origin)
1. Endometrium
2. Anterior pituitary
3. Equine endometrial cups
4. Human syncytiotrophoblast
(A) P-3, Q-4, R-2, S-1
(B) P-4, Q-3, R-1, S-2
(C) P-2, Q-1, R-4, S-3
(D) P-1, Q-2, R-3, S-4

2018

**70.** Which statement INCORRECTLY characterizes the use of sequential media in embryo culture?
(A) It mimics the changing environment of the female reproductive tract
(B) It requires moving embryos to a new drop of medium at specific developmental stages
(C) It provides the exact same nutrients from Day 1 to Day 7
(D) Early media usually have lower glucose concentrations than late media

2004

**71.** What is the primary purpose of adding heparin to in vitro fertilization media for bovine gametes?
(A) To prevent blood clotting in the media
(B) To dissolve the zona pellucida
(C) To stain dead sperm cells
(D) To induce sperm capacitation

2025

**72.** Which embryo stage is most commonly preferred for non-surgical transfer in cattle?
(A) Morula or blastocyst
(B) Early 2-cell stage
(C) Cleaved 8-cell stage
(D) Late hatched blastocyst

2011

**73.** Consider the following statements about H-Y antigen immunological assays:
I. They utilize antibodies against a male-specific antigen.
II. They require the complete destruction of the embryo.

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(B) Capacitation - Occurs in the epididymis
(C) Cortical reaction - Prevents polyspermy
(D) Acrosome reaction - Release of hyalidation and acrosin

2023

**128.** What is the primary purpose of adding serum to in vitro culture media?
(A) To provide a strictly defined, fully synthetic nutrient profile
(B) To completely and reliably suppress mitotic cell divisions
(C) To actively induce highly targeted genetic mutations in vitro
(D) To supply proteins, growth factors, and vitamins

2024

**129.** Match the micro-assisted fertilization techniques (List I) with their mechanical action (List II).
List I: (Micro-assisted fertilization techniques)
P. ICSI
Q. PZD
R. SUZI
S. Enucleation
List II: (Mechanical action)
1. Slicing the zona pellucida to aid sperm entry
2. Injecting sperm into the perivitelline space
3. Injecting sperm directly into the ooplasm
4. Removing the oocyte's chromatin
(A) P-3, Q-1, R-2, S-4
(B) P-3, Q-2, R-1, S-4
(C) P-1, Q-3, R-2, S-4
(D) P-2, Q-4, R-1, S-3

2024

**130.** Consider the following statements regarding telomere dynamics in cloned animals:
I. Cloned animals always possess telomeres longer than naturally conceived animals.
II. Shortened telomeres in clones can lead to premature aging phenotypes.
III. Telomerase activity during early embryogenesis can restore telomere length.
IV. Telomeres are specific DNA sequences at the ends of chromosomes.
V. SCNT completely guarantees perfect telomere restoration.
Which of the above statements are correct?
(A) II, III and IV
(B) I, II and III
(C) III, IV and V
(D) II, IV and V

2022

**131.** Which of the following describes an advantage of single-step media over sequential media for embryo culture?
(A) It allows embryos to auto-condition their environment undisturbed
(B) It requires frequent handling and disruption of the culture environment
(C) It provides shifting glucose concentrations matching in vivo conditions
(D) It actively eliminates metabolic waste products automatically

2016

**132.** Analyze the given assertion and reason, then select the correct option:
**Assertion (A):** The generation of iPSCs eliminates the ethical concerns associated with using embryonic stem cells.
**Reason (R):** iPSCs are derived from adult somatic cells rather than by destroying pre-implantation embryos.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

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2011

**133.** What is the primary purpose of action of melengestrol acetate (MGA) in cattle estrus synchronization?
(A) It causes acute luteolysis and lyses the corpus luteum
(B) An oral progestin to suppress estrus
(C) It induces a sudden massive LH surge immediately upon ingestion
(D) It directly and rapidly stimulates tertiary follicular growth

2017

**134.** Which phenomenon is an expected effect of polyspermy in mammalian fertilization?
(A) Normal, healthy development of dizygotic twins
(B) Greatly enhanced hybrid vigor in the resulting offspring
(C) Polyploidy and embryonic lethality
(D) Spontaneous formation of an unviable haploid embryo

2021

**135.** Identify the correct statement regarding embryo bisection.
(A) A bisected embryo will invariably result in

retrovirus into the host genome
(B) To create targeted double-strand breaks for specific genome editing
(C) To measure the total nuclear DNA content of individual mammalian sperm cells
(D) To synchronize estrus cycles in large herds of domestic livestock animals

2019
**202.** Given below are two statements regarding SCNT: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Epigenetic reprogramming is often incomplete in somatic cell clones.
**Reason (R):** The somatic nucleus has a highly specialized chromatin structure that the oocyte cytoplasm struggles to fully reset to an embryonic state.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2001
**203.** Which is a characteristic feature of an unfertilized metaphase II oocyte?
(A) Extrusion of the first polar body
(B) Presence of two distinct pronuclei
(C) Absence of a zona pellucida
(D) Formation of a blastocoele

2006
**204.** Consider the following statements about stem cell markers:
I. SSEA-1, SSEA-3, and SSEA-4 are surface glycolipids.
II. Nanog is a nuclear transcription factor.
III. Oct-4 is expressed in the trophectoderm but not in the inner cell mass.
IV. The expression of pluripotency markers decreases as cells differentiate.
Which of the above statements is/are INCORRECT?
(A) I and II
(B) III only
(C) IV only
(D) I and IV

2021
**205.** What is the key advantage of using non-permeating cryoprotectants like sucrose during embryo freezing/thawing?
(A) They rapidly penetrate the cell membrane to instantly halt all metabolic activity
(B) They dissolve the glycoprotein matrix of the zona pellucida to allow rapid freezing
(C) They generate massive amounts of intracellular heat to prevent crystallization
(D) They act as an osmotic buffer to safely draw water out of the cell

2010
**206.** Which method is most effective for producing entirely monozygotic twin calves?
(A) Superovulation and artificial insemination of donor cows
(B) Intracytoplasmic sperm injection under high magnification
(C) Micromanipulation and bisection of a single morula
(D) In vitro culture of embryos in double-strength media

2005
**207.** Identify the correct pair regarding embryo transfer.
(A) Flushing medium - Medium containing high concentrations of toxic CPAs
(B) Transfer catheter - Instrument used to deposit embryos into the uterine horn
(C) Foley catheter - Balloon catheter used for surgical removal of embryos
(D) Epidural - Anesthetic drug administered to the recovered embryo directly

2004
**208.** What defines the physiological phenomenon of estrus?
(A) The period of pregnancy maintained by peak progesterone levels
(B) The process of the embryo hatching from the zona pellucida
(C) The physical release of the egg from the ovary
(D) The period of sexual receptivity driven by peak estrogen levels

2005
**209.** Arrange the following reproductive events in the correct temporal sequence for a cow undergoing AI:
I. Ovulation
II. Estrus (standing heat)
III. LH surge
IV. Fertilization
(A) II, III, I, IV
(B) III, II, I, IV
(C) II, I, III, IV

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2002

**144.** Which process does the term "seeding" refer to in cryobiology?
(A) Systematically adding specialized antibiotics to the culture media
(B) Uniformly plating pluripotent stem cells onto an active cellular feeder layer
(C) Mechanically injecting a single selected sperm directly into the oocyte
(D) Inducing extracellular ice formation

2021

**145.** Consider the following statements about PCR-based embryo sexing:
I. It is highly accurate.
II. It requires taking a small biopsy from the embryo.
III. It usually targets Y-chromosome specific sequences like the SRY gene.
IV. It takes several months to obtain a result.
Which of the above statements is/are INCORRECT?
(A) I and II
(B) II and III
(C) IV only
(D) I, II and III

2002

**146.** Arrange the following steps of an IVF protocol in the correct chronological order:
I. Cumulus-oocyte complex grading
II. Follicular aspiration
III. Gamete co-incubation
IV. In vitro maturation
(A) II, I, IV, III
(B) I, II, III, IV
(C) II, IV, I, III
(D) IV, II, I, III

2012

**147.** What is the defining characteristic of a defined culture medium?
(A) All chemical components are known
(B) It contains highly elevated concentrations of crude fetal bovine serum
(C) It actively utilizes a supportive growth layer of somatic feeder cells
(D) It is primarily defined by a total, absolute lack of energy substrates

2015

**148.** Which pharmacological agent is typically used to induce superovulation in cattle?
(A) Posterior pituitary oxytocin
(B) Luteolytic prostaglandin $F_2$ alpha
(C) Anterior pituitary prolactin
(D) Follicle-stimulating hormone

2019

**149.** Match the reproductive structures (List I) with their primary function (List II).
List I: (Reproductive structures)
P. Epididymis
Q. Corpus luteum
R. Zona pellucida
S. Inner cell mass
List II: (Primary function)
1. Prevents polyspermy and protects the early embryo
2. Gives rise to the embryo proper
3. Sperm maturation and storage
4. Progesterone production
(A) P-3, Q-4, R-1, S-2
(B) P-4, Q-3, R-2, S-1
(C) P-2, Q-1, R-4, S-3
(D) P-1, Q-2, R-3, S-4

2012

**150.** Analyze the given assertion and reason, then select the correct option:
**Assertion (A):** Mesenchymal stem cells are frequently used in veterinary medicine to treat equine tendon injuries.
**Reason (R):** They possess anti-inflammatory properties and can differentiate into tenocytes to promote tissue repair.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

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2019

**151.** Which factor is considered a disadvantage of using eCG for superovulation?
(A) It requires only a single injection which simplifies the administration protocol
(B) It is significantly less expensive and more easily sourced than purified FSH
(C) It completely fails to stimulate follicular development and growth in sheep
(D) It causes a high incidence of cystic follicles due to its long halF-life

2018

**152.** Identify the correct statement regarding embryo grading standards.
(A) IETS guidelines are universally applied for grading livestock embryos
(B) Embryos with 30% fragmentation are

male-female twins
(B) Embryo bisection is commonly performed on unfertilized oocytes
(C) Embryo bisection requires the use of CRISPR-Cas9
(D) The two halves of a bisected embryo are genetically identical

2015
**136.** What is the consequence of failing to remove cryoprotectants from a thawed embryo prior to transfer?
(A) Osmotic shock and cellular toxicity
(B) The thawed embryo will spontaneously and immediately hatch
(C) The embryo will immediately multiply into identical quadruplets
(D) The protective outer zona pellucida will dissolve harmlessly

2009
**137.** Consider the following statements about the generation of monozygotic twins:
I. They can occur naturally via the splitting of the inner cell mass.
II. They can be produced artificially via embryo bisection.
III. They always share the same gender.
Which of the above statements are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I, II and III

2005
**138.** Which technique evaluates DNA content differences to sort X and Y sperm?
(A) Agarose gel macromolecule electrophoresis
(B) Flow cytometry using Hoechst dye
(C) Standard genomic DNA Southern blotting
(D) Somatic cell nuclear transfer cloning

2012
**139.** Match the stages of embryo development (List I) with their expected day of occurrence post-estrus in cattle (List II).
List I: (Stages of embryo development)
P. 2-cell stage
Q. Morula
R. Blastocyst
S. Hatched Blastocyst
List II: (Expected day of occurrence post-estrus in cattle)
1. Day 7-8
2. Day 9-10
3. Day 5-6
4. Day 1-2
(A) P-4, Q-3, R-1, S-2
(B) P-3, Q-4, R-2, S-1
(C) P-1, Q-2, R-4, S-3
(D) P-2, Q-1, R-3, S-4

2010
**140.** Which of the following is NOT a recognized consequence of the Large Offspring Syndrome?
(A) Significantly increased incidence of abortion
(B) Complex macro and micro placental abnormalities
(C) Unusually long animal lifespan
(D) Markedly enlarged internal vascular organs

2010
**141.** Analyze the given assertion and reason, then select the correct option:
**Assertion (A):** Random integration via pronuclear microinjection often results in variable expression levels of the transgene.
**Reason (R):** The position effect variegation heavily influences transgene expression depending on where it integrates in the host genome.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2023
**142.** What role does Oct-4 play in embryonic stem cells?
(A) It acts as a major structural protein component of the cellular cytoskeleton
(B) It serves as an active cellular surface receptor facilitating viral entry
(C) Transcription factor maintaining pluripotency
(D) It actively degrades and recycles damaged intracellular proteins

2005
**143.** Identify the INCORRECT statement regarding the biological challenges in cloning.
(A) Clones frequently exhibit highly abnormal and aberrant DNA methylation profiles
(B) Somatic cell cloning is highly efficient
(C) Mitochondrial heteroplasmy regularly occurs when donor and recipient organelles mix
(D) Proper temporal synchronization of the donor somatic cell cycle is critical

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(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2021
**161.** Which technique utilizes an electrical pulse to merge the donor cell with the enucleated oocyte during SCNT?
(A) Electrofusion
(B) Microinjection
(C) Electroporation
(D) Transfection

2010
**162.** Consider the following statements about transgenesis in livestock:
I. It can be used to improve disease resistance.
II. It requires the integration of foreign DNA into the host genome.
III. It guarantees that all offspring of the transgenic founder will express the gene identically.
IV. It can utilize retroviral vectors.
Which of the above statements is/are INCORRECT?
(A) I and II
(B) II and III
(C) III only
(D) I and IV

2012
**163.** What describes the concept of 'totipotency'?
(A) The ability to divide and produce all differentiated cells, including extraembryonic tissues
(B) The ability of a stem cell to differentiate into only a few specific, closely related cell types
(C) The restricted developmental ability of a cell to form only mesoderm-derived tissues and organs
(D) The absolute inability of a highly specialized cell to undergo mitotic division or replication

2012
**164.** Which marker is heavily associated with the maintenance of pluripotency in ESCs?
(A) Caspase-3
(B) MyoD
(C) Amylase
(D) Oct-4

2008
**165.** Arrange the following techniques in the order of their historical development (earliest to latest):
I. Artificial Insemination
II. Somatic Cell Nuclear Transfer
III. CRISPR-Cas9 Genome Editing
IV. In Vitro Fertilization
(A) I, IV, II, III
(B) I, II, IV, III
(C) IV, I, III, II
(D) II, I, IV, III

2025
**166.** What primarily drives the physical process of blastocyst hatching?
(A) Decreased internal pressure within the blastocoele
(B) High levels of progesterone acting on the zona pellucida
(C) Rhythmic expansion and contraction of the blastocyst
(D) The physical action of the sperm tail

2023
**167.** Identify the correct pair regarding embryo developmental staging.
(A) Morula - Fluid-filled cavity stage
(B) Blastocyst - Solid ball of cells
(C) Oocyte - Post-implantation stage
(D) Zygote - Single cell formed by syngamy

2005
**168.** Which of the following describes a permeating cryoprotectant?
(A) A small molecule like glycerol that crosses the cell membrane
(B) A large, high-molecular-weight polymer that remains entirely extracellular
(C) A specialized membrane protein that actively pumps water out of the cytoplasm
(D) A highly hydrophobic lipid molecule that solidifies the outer cell wall structure

NODIA

2003
**169.** Match the SCNT terms in List I with their definitions in List II.
List I: (SCNT Terms)
P. Enucleation
Q. Reconstruction
R. Electrofusion
S. Activation
List II: (Definitions)
1. Introduction of the somatic cell into the perivitelline space
2. Removal of the oocyte's nuclear material
3. Inducing embryonic development via calcium transients
4. Merging the somatic cell with the oocyte cytoplasm

stage
(D) It makes the resulting culture media completely transparent and difficult to visualize

2007
**186.** Given below are two statements regarding embryo evaluation: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** In the IETS grading system, Grade 1 embryos are classified as excellent or outstanding quality.
**Reason (R):** Grade 1 embryos exhibit symmetrical blastomeres and have less than 10% cellular fragmentation.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2002
**187.** What is the fundamental function of the acrosome reaction?
(A) To release hydrolytic enzymes that digest the zona pellucida
(B) To physically propel the sperm through the female tract
(C) To prevent other sperm from binding to the oocyte
(D) To eject the male pronucleus into the perivitelline space

2019
**188.** Match the assisted reproductive technologies in List I with their primary limitation in List II.
List I: (Assisted Reproductive Technologies)
P. Conventional IVF
Q. SCNT (Cloning)
R. Pronuclear Microinjection
S. Flow Cytometry (Semen Sexing)
List II: (Primary Limitation)
1. Low sorting speed and potential sperm damage
2. High incidence of epigenetic abnormalities and Large Offspring Syndrome
3. Random integration and variable transgene expression
4. Unpredictable rates of polyspermy
(A) P-4, Q-2, R-3, S-1
(B) P-2, Q-3, R-1, S-4
(C) P-3, Q-1, R-4, S-2
(D) P-1, Q-4, R-2, S-3

2024
**189.** Consider the following statements about gene editing using CRISPR-Cas9:
I. It induces targeted double-strand DNA breaks.
II. It requires a synthetic guide RNA (sgRNA) matching the target sequence.
III. It relies entirely on random viral integration.
IV. It can be used for both gene knockouts and precise gene insertions.
Which of the above statements is/are correct?
(A) I, II and IV
(B) I, II and III
(C) II, III and IV
(D) I, III and IV

2017
**190.** Which structural characteristic primarily defines the blastocyst stage?
(A) A highly compact, solid sphere of completely identical, undifferentiated blastomeres
(B) The extrusion of two polar bodies and the subsequent formation of two distinct pronuclei
(C) Differentiation into an inner cell mass and a trophectoderm layer surrounding a fluid cavity
(D) The complete dissolution of the glycoprotein-based zona pellucida during early hatching

2025
**191.** Identify the INCORRECT statement regarding the handling of embryos during MOET.
(A) Embryos must be kept at physiological temperatures (approx. 37°C) during searching and evaluation
(B) Prolonged exposure to ultraviolet light is harmless to embryos
(C) Specialized flushing media containing a protein source is utilized
(D) Extreme pH fluctuations in the media can cause embryo death

2015
**192.** What is the primary role of a cryoprotectant like DMSO during the freezing process?
(A) To initiate immediate cell division and metabolic activity during thawing
(B) To completely dehydrate the outer cell wall and prevent any water influx
(C) To temporarily dissolve the zona pellucida for easier molecular penetration
(D) To prevent mechanical damage from intracellular ice crystal formation

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3. Induces ovulation or luteinization of a dominant follicle
4. Stimulates follicular growth

(A) P-3, Q-1, R-2, S-4
(B) P-1, Q-2, R-3, S-4
(C) P-4, Q-3, R-1, S-2
(D) P-2, Q-4, R-3, S-1

2016

**178.** Given below are two statements regarding gamete biology: one is labelled as Assertion (A) and the other is labelled as Reason (R).

**Assertion (A):** Capacitation is a reversible process.

**Reason (R):** Decapacitation factors present in seminal plasma can re-bind to the sperm surface to reverse the capacitated state.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2009

**179.** What distinguishes cleavage divisions from typical somatic cell mitosis?

(A) Cleavage involves cell divisions without any increase in total cytoplasmic mass
(B) Cleavage requires massive cellular growth of the cytoplasm before each consecutive division
(C) Cleavage only occurs in unfertilized metaphase II oocytes during parthenogenetic activation
(D) Cleavage generates genetically diverse daughter cells due to active meiotic recombination events

2017

**180.** Consider the following statements regarding Cumulus-Oocyte Complexes (COCs) during IVF:

I. Cumulus cells hinder sperm penetration and must be completely removed prior to co-incubation.
II. Cumulus cells secrete factors that attract and capacitate sperm.
III. Expanded cumulus investments indicate maturation.
IV. Intact COCs generally yield higher fertilization rates than denuded oocytes.

Which of the above statements is/are INCORRECT?

(A) I only
(B) II and III
(C) III and IV
(D) I and IV

2012

**181.** Which biological agent is a key component of the innate polyspermy block in mammalian oocytes?

(A) Acrosin
(B) Cortical granules
(C) Polar bodies
(D) Cumulus cells

2017

**182.** Identify the correct pair representing a stem cell type and its origin.

(A) Mesenchymal stem cells - Neural tube
(B) iPSCs - Unfertilized eggs
(C) Embryonic stem cells - Inner cell mass
(D) Spermatogonial stem cells - Ovaries

2007

**183.** Arrange the following steps of a typical superovulation protocol in cattle in the correct order:

I. Flushing of embryos
II. Onset of estrus and artificial insemination
III. Multiple injections of FSH
IV. Injection of Prostaglandin $F_2$ alpha

(A) III, IV, II, I
(B) IV, III, II, I
(C) III, II, IV, I
(D) II, IV, III, I

2021

**184.** What defines a 'bioreactor' animal in the context of gene pharming?

(A) An animal engineered to produce human pharmaceuticals, typically in its milk
(B) An experimental research animal that digests feed entirely in an artificial rumen setup
(C) A donor animal strictly used to generate pluripotent embryonic stem cells in vitro
(D) A somatic cell clone that produces highly excessive amounts of endogenous growth hormone

2022

**185.** Which is the most significant disadvantage of using fetal bovine serum in in vitro culture media?

(A) It provides essential growth factors and hormones that promote early cell division
(B) It introduces undefined variables and carries a risk of viral contamination
(C) It forces the developing embryo to hatch prematurely before reaching the blastocyst

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(A) P-2, Q-1, R-4, S-3
(B) P-1, Q-2, R-3, S-4
(C) P-3, Q-4, R-1, S-2
(D) P-4, Q-3, R-2, S-1

2002

**170.** Given below are two statements regarding embryo flushing techniques: one is labelled as Assertion (A) and the other is labelled as Reason (R).

**Assertion (A):** A local epidural anesthetic is typically administered to the donor cow prior to non-surgical flushing.

**Reason (R):** Epidural anesthesia relaxes the rectum and prevents straining, facilitating rectal palpation and catheter manipulation.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2002

**171.** What is the fundamental principle of using a CIDR device in estrus synchronization?

(A) It provides a continuous release of progesterone, mimicking the luteal phase
(B) It releases massive doses of synthetic estrogen to induce immediate standing estrus behavior
(C) It directly lyses the functional corpus luteum to initiate rapid luteolysis in the animal
(D) It stimulates endogenous FSH production in the pituitary gland to grow a cohort of follicles

2012

**172.** Consider the following statements about stem cell types:

I. Embryonic stem cells are pluripotent.
II. Adult stem cells are generally multipotent.
III. iPSCs behave similarly to embryonic stem cells.
IV. Unipotent cells can differentiate into any cell type.

Which of the above statements is/are correct?

(A) I, II and III
(B) II, III and IV
(C) I, III and IV
(D) I, II and IV

2018

**173.** Which consequence is associated with incomplete epigenetic reprogramming during cloning?

(A) The birth of perfectly normal, healthy offspring possessing highly superior production genetics
(B) Enhanced immune system function and resistance to common infectious diseases
(C) The automatic generation of monozygotic twins from a single reconstructed embryo
(D) Extremely high rates of fetal loss and severe placental abnormalities during gestation

NODIA

2010

**174.** Identify the INCORRECT statement concerning transgenic livestock production.

(A) Pronuclear microinjection has a relatively low efficiency of stable integration
(B) Transgenes can insert randomly, causing insertional mutagenesis
(C) Retroviral vectors only target specific, pre-determined loci in the host genome
(D) Somatic Cell Nuclear Transfer guarantees 100% stable integration if the donor cell is successfully transfected

2025

**175.** What represents the primary limitation of using sequential media for in vitro embryo culture?

(A) It completely fails to mimic the dynamic environment of the female reproductive tract
(B) It requires moving embryos, exposing them to environmental stress
(C) It contains high levels of toxic heavy metals that damage developing blastomeres
(D) It permanently prevents the formation of a fluid-filled blastocoele cavity

2005

**176.** Which technique is essentially required to confirm the success of a gene knockout in a livestock embryo?

(A) Visual inspection under a light microscope
(B) Staining with a DNA-specific dye
(C) Evaluating the embryo's swimming speed
(D) Molecular screening via PCR and sequencing

2005

**177.** Match the estrus synchronization agents in List I with their primary function in List II.

List I: (Estrus Synchronization Agents)

P. GnRH
Q. Prostaglandin $F_2$ alpha
R. Progestin (CIDR)
S. eCG

List II: (Primary Function)

1. Causes regression of the corpus luteum
2. Suppresses estrus and ovulation

2015

**193.** Given below are two statements regarding sperm capacitation: one is labelled as Assertion (A) and the other is labelled as Reason (R).

**Assertion (A):** Capacitated sperm display hyperactivated motility.

**Reason (R):** Hyperactivation provides the mechanical force necessary to penetrate the cumulus oophorus and zona pellucida.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2009

**194.** Which hormone is released by the embryo in sheep and cattle to prevent luteolysis (maternal recognition of pregnancy)?

(A) Human Chorionic Gonadotropin (hCG)
(B) Prostaglandin $F_2$ alpha
(C) Interferon-tau
(D) Estradiol

2023

**195.** Consider the following statements regarding the production of transgenic animals:

I. Sperm-mediated gene transfer is widely considered the most reliable and efficient method.
II. Pronuclear microinjection has a low rate of successful integration (often $<5\%$).
III. SCNT using transfected somatic cells ensures that all resulting clones carry the transgene.

Which of the above statements is/are correct?

(A) I and II
(B) II and III
(C) I and III
(D) I, II and III

2009

**196.** Arrange the following steps of an embryo freezing protocol using a slow-freezing method in the correct chronological order:

I. Addition of cryoprotectant (e.g., glycerol)
II. Seeding to induce extracellular ice formation
III. Cooling to -30°C at a controlled rate
IV. Plunging into liquid nitrogen

(A) I, II, III, IV
(B) II, I, III, IV
(C) I, III, II, IV
(D) III, II, I, IV

2021

**197.** What is the biological function of the trophectoderm layer in a mammalian blastocyst?

(A) It forms the entire nervous system of the fetus
(B) It forms the fetal portion of the placenta
(C) It directly generates embryonic stem cells
(D) It develops into the primordial germ cells

2023

**198.** Which concept involves replacing the nucleus of an unfertilized oocyte with the nucleus of a specialized somatic cell?

(A) Pronuclear microinjection
(B) In vitro maturation
(C) Somatic Cell Nuclear Transfer
(D) Embryo splitting

2021

**199.** Match the cryobiology terms in List I with their fundamental mechanisms in List II.

List I: (Cryobiology Terms)
P. Slow freezing
Q. Vitrification
R. Seeding
S. Thawing

List II: (Fundamental Mechanisms)
1. Transitioning from a solid to a liquid state
2. Inducing controlled ice formation to avoid supercooling
3. Ultra-rapid cooling achieving a glass-like state without ice
4. Gradual dehydration of cells driven by extracellular ice

(A) P-4, Q-3, R-2, S-1
(B) P-3, Q-4, R-1, S-2
(C) P-2, Q-1, R-4, S-3
(D) P-1, Q-2, R-3, S-4

2007

**200.** Identify the correct sequence of media used to culture an embryo from zygote to hatched blastocyst.

(A) Cleavage medium (Day 1-3) followed by Blastocyst medium (Day 4-8)
(B) Maturation medium (Day 1-3) followed by Capacitation medium (Day 4-8)
(C) Freezing medium (Day 1-3) followed by Thawing medium (Day 4-8)
(D) Blastocyst medium (Day 1-3) followed by Cleavage medium (Day 4-8)

2013

**201.** What is the primary purpose of using zinc finger nucleases (ZFNs) in livestock biotechnology?

(A) To randomly insert a replication-deficient

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generally classified as Grade 1
(C) Embryo diameter remains the sole morphological criterion used for grading
(D) Grade 4 embryos are considered the most ideal selection for cryopreservation

2006
**153.** What is the primary purpose of the Ovsynch protocol in dairy cattle?
(A) To induce superovulation and increase the recovery rate of viable embryos
(B) To synchronize ovulation to allow for fixed-time artificial insemination
(C) To prepare donor cows for non-surgical embryo flushing and transfer
(D) To treat cystic ovarian disorders permanently and restore cyclicity

2001
**154.** Consider the following statements about DNA content in sperm:
I. Mammalian Y-bearing sperm contain more DNA than X-bearing sperm.
II. Flow cytometry utilizes this difference to sort sperm.
III. The difference in DNA content is roughly 3-4% in cattle.
Which of the above statements is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I, II and III

2009
**155.** Which of the following represents an advantage of ICSI over standard IVF?
(A) ICSI allows fertilization using severe oligozoospermic or dead sperm
(B) ICSI is technically less demanding and requires no specialized laboratory equipment
(C) ICSI relies entirely on natural sperm selection based on individual motility
(D) ICSI completely prevents the inheritance of paternal genetic abnormalities

2012
**156.** What distinguishes a vitrification protocol from a slow-freezing protocol?
(A) Vitrification uses significantly lower concentrations of permeating cryoprotectants
(B) Vitrification employs ultra-rapid cooling to achieve an ice-free glass-like state
(C) Vitrification requires a sophisticated, highly programmable biological freezing machine
(D) Vitrification takes several hours of stepwise incubation steps to complete fully

2010
**157.** Match the reproductive hormones in List I with their primary source in List II.
List I: (Reproductive Hormones)
P. Estradiol
Q. FSH
R. Progesterone
S. Interferon-tau
List II: (Primary Source)
1. Anterior Pituitary
2. Corpus Luteum
3. Granulosa cells of the follicle
4. Trophoblast of the conceptus
(A) P-3, Q-1, R-2, S-4
(B) P-1, Q-3, R-4, S-2
(C) P-2, Q-4, R-1, S-3
(D) P-4, Q-2, R-3, S-1

2016
**158.** Identify the INCORRECT statement regarding Cumulus-Oocyte Complexes (COCs).
(A) The cumulus cells supply the oocyte with essential developmental metabolites
(B) Expansion of the cumulus mass is a highly reliable sign of oocyte maturation
(C) Gap junctions directly connect the surrounding cumulus cells to the oocyte
(D) Denuded oocytes mature much faster and better in vitro than intact COCs

2009
**159.** What is the main cause of polyspermy block failure during in vitro fertilization?
(A) Using highly capacitated sperm cells prepared via standard heparin protocols
(B) Extremely high sperm concentrations in the co-incubation drop
(C) Supplementing the culture media with high concentrations of bovine serum albumin
(D) Using single-step culture media instead of sequential media formulations

2022
**160.** Given below are two statements regarding gene editing tools: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** CRISPR-Cas9 is widely preferred over Zinc Finger Nucleases for genome editing.
**Reason (R):** CRISPR-Cas9 target specificity is determined by a simple guide RNA rather than complex protein engineering.
(A) Both A and R are true and R is the correct explanation of A.

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(D) III, I, II, IV

2003

**210.** Match the applications of transgenic technology in List I with examples in livestock in List II.

List I: (Applications of Transgenic Technology)

P. Enhanced production traits
Q. Gene pharming
R. Disease resistance
S. Environmental mitigation

List II: (Examples in Livestock)

1. Pigs expressing phytase to reduce phosphorus in manure
2. Goats producing human antithrombin in milk
3. Cattle engineered for resistance to mastitis
4. Sheep engineered for enhanced wool growth

(A) P-4, Q-2, R-3, S-1
(B) P-2, Q-4, R-1, S-3
(C) P-3, Q-1, R-2, S-4
(D) P-1, Q-3, R-4, S-2

2018

**211.** Given below are two statements regarding follicular dynamics: one is labelled as Assertion (A) and the other is labelled as Reason (R).

**Assertion (A):** During the luteal phase, a dominant follicle will emerge but ultimately undergo atresia.

**Reason (R):** High levels of progesterone suppress the LH surge required for ovulation.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2019

**212.** What distinguishes adult stem cells from induced pluripotent stem cells (iPSCs)?

(A) Adult stem cells have a higher differentiation potential and can form all embryonal tissues compared to iPSCs
(B) Adult stem cells form multi-lineage teratomas much more easily when injected into immune-deficient host mice
(C) Adult stem cells completely lack any recognizable surface markers used for cell sorting and characterization
(D) Adult stem cells are multipotent and naturally occurring, while iPSCs are reprogrammed to be pluripotent

2009

**213.** Consider the following statements regarding sex-sorted semen:

I. Sorting efficiency is typically around 90-95% for the desired sex.
II. Sorting significantly increases the lifespan and fertility of the sperm.
III. Sorting requires the use of a DNA-binding fluorochrome.
IV. Sex-sorted semen generally yields lower conception rates compared to conventional semen.

Which of the above statements is/are correct?

(A) I, II and III
(B) I, III and IV
(C) II, III and IV
(D) I, II and IV

2024

**214.** Which statement correctly characterizes the zona pellucida?

(A) It is a highly cellular, protective cellular layer completely surrounding the mammalian oocyte
(B) It forms immediately after fertilization as a new membrane layer to block subsequent polyspermy
(C) It is an acellular glycoprotein matrix essential for species-specific sperm binding
(D) It dissolves automatically and instantaneously once the first sperm cell penetrates through it

2019

**215.** What is the primary disadvantage of using pronuclear microinjection for transgenesis?

(A) It displays an extremely high and predictable efficiency rate of stable genomic integration in livestock
(B) It is a restricted technique that can only be used to create loss-oF-function gene knockout animals
(C) It strictly requires the complete surgical destruction of the recipient surrogate female during transfer
(D) Integration occurs randomly, leading to variable expression and potential insertional mutagenesis

2021

**216.** Which physiological signal primarily initiates the maternal recognition of pregnancy in pigs?

(A) Secretion of estrogen by the conceptus
(B) Secretion of interferon-tau by the conceptus
(C) Immediate regression of the corpus luteum

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(A) It acts similarly to FSH, stimulating follicular growth
(B) It directly causes the regression of the corpus luteum
(C) It acts similarly to LH, inducing ovulation of mature follicles
(D) It permanently suppresses the estrous cycle

2016

**247.** Identify the correct pair regarding the mechanisms of natural syngamy.
(A) Cortical granules - Intracellular organelles containing structures facilitating sperm motility
(B) ZP3 - Specific glycoprotein receptor protein that blocks polyspermy at the membrane level
(C) Pronucleus - A fully diploid nuclear structure formed immediately prior to syngamy events
(D) Acrosin - Enzyme facilitating penetration of the zona pellucida

2021

**248.** Which factor is heavily associated with the decline in developmental competence of oocytes collected from small, subordinate follicles?
(A) Lack of adequate cytoplasmic maturation and stored maternal RNA
(B) Premature activation and transcription of the embryonic genome
(C) An excessively thick and impermeable outer glycoprotein zona pellucida
(D) Over-expression of core pluripotency transcription factors and markers

2021

**249.** Given below are two statements regarding cryoprotectants: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Trehalose is effectively used as a non-permeating cryoprotectant.
**Reason (R):** Trehalose creates an osmotic gradient that draws water out of the cell, minimizing intracellular ice formation.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2016

**250.** Match the components of an IVF system in List I with their primary function in List II.
List I: (Components of an IVF System)
P. Heparin
Q. BO medium or TALP
R. Mineral oil overlay
S. Carbon dioxide incubator
List II: (Primary Function)
1. Prevents evaporation and temperature fluctuations
2. Maintains physiological pH using a bicarbonate buffer system
3. Acts as a capacitation agent for bovine sperm
4. Provides the basic nutrient environment for co-incubation
(A) P-3, Q-4, R-1, S-2
(B) P-4, Q-3, R-2, S-1
(C) P-1, Q-2, R-3, S-4
(D) P-2, Q-1, R-4, S-3

2008

**251.** What fundamentally occurs during the process of somatic cell nuclear reprogramming?
(A) The somatic epigenetic marks are erased and replaced with embryonic marks
(B) The somatic DNA sequence is actively and permanently altered to match embryonic DNA sequences
(C) The somatic cell membrane is completely dissolved using specific electrical or chemical treatments
(D) The somatic mitochondria completely and rapidly replace the endogenous recipient oocyte mitochondria

2001

**252.** Consider the following statements about gene knockouts:
I. They involve the targeted disruption of a specific gene sequence.
II. They are commonly used to study the function of unknown genes.
III. They guarantee enhanced physical growth in livestock.
IV. They frequently utilize homologous recombination.
Which of the above statements is/are INCORRECT?
(A) I and II
(B) III only
(C) IV only
(D) I and IV

NODIA

2009

**253.** Which best describes the phenomenon of polyspermy block?
(A) The inability of ejaculated sperm to swim

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2022

**224.** Identify the correct pair regarding in vitro embryo production.
(A) IVM - Culturing newly fertilized mammalian embryos directly to the blastocyst stage
(B) IVC - Maturing primary, immature germinal vesicle oocytes into metaphase II oocytes
(C) ICSI - Splitting a high-quality pre-implantation embryo into two identical halves
(D) IVF - Co-incubation of mature fertilizable oocytes and fully capacitated sperm

2003

**225.** Which phenomenon leads to the rejection of somatic cell clones by surrogate mothers?
(A) Aberrant placental development stemming from improper genomic imprinting
(B) Massive production of identical twin fetuses crowding the uterine horns
(C) The developing clone actively attacking the surrogate's maternal immune system
(D) Complete failure of the sperm acrosome reaction during fertilization steps

2005

**226.** Given below are two statements regarding cryoprotectants: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Vitrification media contain significantly higher concentrations of cryoprotectants than slow-freezing media.
**Reason (R):** High cryoprotectant concentrations are required to sufficiently increase viscosity and prevent ice crystal formation during ultra-rapid cooling.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2003

**227.** What biological mechanism is utilized by Partial Zona Dissection (PZD) to assist fertilization?
(A) Mechanical breaching of the zona pellucida to give sperm direct access to the oolemma
(B) Direct microinjection of a single sperm cell into the oocyte nucleus or pronuclei
(C) Dissolving the entire glycoprotein matrix of the zona pellucida using an acid Tyrode's solution
(D) Forcing sperm cells to swim faster toward the oocyte using high-voltage electrical currents

2015

**228.** Consider the following statements regarding the estrous cycle in livestock:
I. The luteal phase is characterized by a mature corpus luteum.
II. Prostaglandin $F_2$ alpha causes the regression of the corpus luteum.
III. The follicular phase is dominated by progesterone.
IV. Estrus coincides with the peak of the LH surge.
Which of the above statements is/are INCORRECT?
(A) I and II
(B) III only
(C) IV only
(D) I and IV

2003

**229.** Arrange the following steps of somatic cell cloning in the correct sequence:
I. Enucleation of recipient oocyte
II. Synchronization of donor somatic cells
III. Activation of reconstructed embryo
IV. Electrofusion
(A) II, I, IV, III
(B) I, II, III, IV
(C) II, IV, I, III
(D) I, IV, II, III

2001

**230.** Match the markers in List I with their associated stem cell property or type in List II.
List I: (Markers)
P. Oct-4
Q. CD44 / CD90
R. Alkaline Phosphatase
S. MyoD
List II: (Associated Stem Cell Property or Type)
1. Mesenchymal stem cell surface markers
2. Transcription factor critical for pluripotency
3. Muscle lineage commitment marker
4. Enzyme heavily expressed in undifferentiated ES cells
(A) P-2, Q-1, R-4, S-3
(B) P-1, Q-2, R-3, S-4
(C) P-4, Q-3, R-1, S-2
(D) P-2, Q-4, R-3, S-1

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(A) I, II and IV
(B) I, III and IV
(C) II, III and IV
(D) I, II and III

2021

**261.** What is the fundamental difference between sequential media and single-step media in embryo culture?
(A) Sequential media are purely synthetic, while single-step media contain heavy serum additions and undefined proteins
(B) Sequential media require changing formulations, while single-step media use one continuous formulation
(C) Sequential media only support early cleavage stages, while single-step media only support late blastulation stages
(D) Sequential media prevent the embryo from hatching, while single-step media naturally induce hatching from the zona

2011

**262.** Which method allows for the highest efficiency of targeted double-strand breaks at highly specific genomic loci?
(A) Pronuclear microinjection
(B) Sperm-mediated gene transfer
(C) CRISPR-Cas9
(D) Retroviral vector integration

2015

**263.** Identify the INCORRECT statement regarding the application of ES cells in livestock.
(A) They possess the theoretical potential to differentiate into any cell type of the adult body tissues
(B) They hold massive promise for generating advanced transgenic models and gene-edited livestock strains
(C) They require highly specialized culture conditions to prevent spontaneous and unwanted differentiation
(D) True, validated ES cells have been remarkably easy to derive and maintain in ungulates compared to mice

2017

**264.** Given below are two statements regarding estrus synchronization: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Progestin-based synchronization protocols often yield highly synchronous estrus but lower initial conception rates.
**Reason (R):** Prolonged artificial progestin exposure can cause the ovulation of an aged, less competent follicle.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2005

**265.** What represents the primary advantage of utilizing iPSCs over ESCs in clinical applications?
(A) iPSCs naturally grow much faster and require significantly fewer metabolic nutrients during standard in vitro culture
(B) iPSCs circumvent ethical concerns of embryo destruction and allow for autologous therapies
(C) iPSCs absolutely cannot form benign or malignant teratomas when transplanted into recipient host tissues in vivo
(D) iPSCs are completely resistant to all known viral infections and bacterial contaminations during cell passage

2013

**266.** Arrange the following steps of an embryo transfer procedure in cattle in the correct sequence:
I. Administration of epidural anesthesia
II. Rectal palpation to locate the corpus luteum
III. Loading the embryo into the transfer gun
IV. Depositing the embryo into the ipsilateral uterine horn
(A) III, I, II, IV
(B) I, II, III, IV
(C) I, III, II, IV
(D) III, II, I, IV

2010

**267.** Match the stages of follicle development in List I with their defining characteristic in List II.
List I: (Stages of Follicle Development)
P. Primordial follicle
Q. Primary follicle
R. Antral follicle
S. Graafian follicle
List II: (Defining Characteristic)
1. Features a fully developed fluid-filled cavity and is ready for ovulation
2. Oocyte surrounded by a single layer of squamous cells
3. Oocyte surrounded by a single layer of cuboidal cells
4. Characterized by the initial formation of a fluid-filled cavity (antrum)

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I. Compaction
II. Syngamy
III. Hatching
IV. Blastulation
(A) II, I, IV, III
(B) II, IV, I, III
(C) I, II, IV, III
(D) II, I, III, IV

2013

**240.** Match the assisted reproductive technologies in List I with the cellular structure they manipulate in List II.
List I: (Assisted Reproductive Technologies)
P. SCNT
Q. ICSI
R. Pronuclear microinjection
S. PZD
List II: (Cellular Structure Manipulated)
1. The male and female pronuclei of a zygote
2. The entire ooplasm and somatic nucleus
3. The zona pellucida of an unfertilized oocyte
4. The ooplasm and a single spermatozoon
(A) P-2, Q-4, R-1, S-3
(B) P-4, Q-2, R-3, S-1
(C) P-1, Q-3, R-2, S-4
(D) P-3, Q-1, R-4, S-2

2008

**241.** Which best describes the principle of 'competitive exclusion' in the context of the dominant follicle?
(A) The dominant follicle secretes inhibin, depressing FSH levels and starving smaller follicles
(B) The dominant follicle actively competes with the adjacent active corpus luteum for ovarian blood supply
(C) The dominant follicle rapidly absorbs all available free estrogen molecules circulating within the ovary
(D) The dominant follicle physically expands and mechanically destroys smaller neighboring primordial follicles

2003

**242.** Given below are two statements regarding induced pluripotent stem cells (iPSCs): one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** The generation of iPSCs typically utilizes viral vectors to introduce reprogramming factors.
**Reason (R):** Viral vectors pose a risk of insertional mutagenesis, limiting the clinical application of iPSCs.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

NODIA

2013

**243.** What is the fundamental purpose of 'seeding' during slow-rate embryo freezing?
(A) To prevent supercooling by inducing controlled extracellular ice formation
(B) To completely dehydrate the embryo instantly using highly concentrated sugars
(C) To dissolve the glycoprotein matrix of the zona pellucida for CPA penetration
(D) To warm the embryo slightly before final plunging into liquid nitrogen tanks

2024

**244.** Consider the following statements about embryo splitting:
I. Splitting is most successfully performed at the morula or blastocyst stage.
II. Splitting requires a complex micromanipulator setup.
III. Splitting an embryo guarantees two live births.
IV. The splitting blade must be precisely positioned to divide the inner cell mass evenly.
Which of the above statements is/are INCORRECT?
(A) I and II
(B) III only
(C) IV only
(D) I and IV

2025

**245.** Which of the following defines a 'chimeric' animal?
(A) An animal produced entirely by parthenogenetic activation of unfertilized oocytes
(B) An animal containing populations of cells derived from different zygotes
(C) An animal that has been completely stripped of its functional adaptive immune system
(D) An animal that possesses entirely synthetic DNA sequences synthesized in a laboratory

2002

**246.** What biological effect does the administration of human chorionic gonadotropin (hCG) have in livestock?

(D) Massive release of oxytocin

2013

**217.** Identify the INCORRECT statement regarding the application of stem cells in veterinary medicine.
(A) Mesenchymal stem cells can be successfully derived from adipose tissue and bone marrow aspirates
(B) Embryonic stem cells are currently the routine standard of care in all commercial livestock operations
(C) Stem cell therapies are frequently and successfully utilized for treating chronic osteoarthritis in dogs
(D) Induced pluripotent stem cells hold massive potential for creating in vitro canine disease models

2015

**218.** Given below are two statements regarding embryo evaluation: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Compact morulae are easier to evaluate and grade than early cleavage stage embryos.
**Reason (R):** Blastomeres in a compact morula have coalesced into a single tight mass, masking severe individual cellular fragmentation.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2014

**219.** What is the fundamental mechanism driving the separation of X and Y sperm in a flow cytometer?
(A) Deflection of charged droplets containing individual sperm based on their relative fluorescence
(B) A high-intensity magnetic field pulling on the heavier chromatin content of the X chromosome
(C) Density gradient centrifugation forcing the heavier X-bearing sperm to the bottom of the tube
(D) Selective chemical destruction of the Y-bearing sperm population using monoclonal antibodies

2002

**220.** Match the genome editing techniques in List I with their defining characteristic in List II.
List I: (Genome Editing Techniques)
P. Gene Knockout
Q. Gene Knock-in
R. CRISPR-Cas9
S. Retroviral Integration
List II: (Defining Characteristic)
1. Utilizing RNA guidance for targeted cleavage
2. Disruption or deletion of an existing gene
3. Random insertion heavily biased towards active genes
4. Targeted insertion of a novel genetic sequence
(A) P-2, Q-4, R-1, S-3
(B) P-1, Q-2, R-3, S-4
(C) P-3, Q-1, R-4, S-2
(D) P-4, Q-3, R-2, S-1

2008

**221.** Consider the following statements about early cleavage:
I. The total mass of the embryo decreases slightly during early cleavage divisions.
II. Cleavage relies heavily on maternally derived mRNA.
III. The transition from maternal to embryonic genome control occurs immediately after syngamy.
Which of the above statements is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I, II and III

2017

**222.** Which factor is most directly responsible for the induction of the acrosome reaction in vivo?
(A) Exposure to high levels of progesterone in the uterus
(B) The beating of the oviductal cilia
(C) Binding of the sperm to the ZP3 glycoprotein
(D) Capacitation agents in the epididymis

2024

**223.** What role does the beta-lactoglobulin promoter play in the design of an expression vector for gene pharming?
(A) It causes the therapeutic transgene to be ubiquitously expressed in all somatic tissues of the clone
(B) It directs the expression of the transgene specifically to the mammary gland during lactation
(C) It silences the host's native milk protein genes to prevent any cross-contamination of the product
(D) It acts as a primary selectable marker

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through thick cervical mucus secretions in the cervix
(B) The immediate immunological rejection of the motile sperm cells by the surrounding cumulus cells
(C) The physical barricading and closure of the oviductal lumen to prevent further sperm transport
(D) A cascade of events modifying the zona pellucida and oolemma to prevent multiple sperm entry

2009

**254.** Arrange the sequence of steps for generating a transgenic animal via pronuclear microinjection in the correct order:
I. Collection of fertilized zygotes
II. Microinjection of DNA into the male pronucleus
III. Superovulation and mating of donor females
IV. Transfer of injected embryos into a recipient female
(A) III, I, II, IV
(B) I, II, III, IV
(C) III, II, I, IV
(D) I, III, II, IV

2001

**255.** What defines a unipotent stem cell?
(A) It can form any cell type within a specific germ layer during early development
(B) It can only form one specific cell type, though it retains the ability to selF-renew
(C) It is a highly specialized somatic cell that cannot undergo mitotic division at all
(D) It possesses the unique developmental capacity to form a complete living organism

2008

**256.** Identify the correct statement regarding embryo sexing via PCR.
(A) It relies on visual identification of the X and Y chromosomes under a light microscope
(B) It strictly requires the complete chemical destruction of the entire embryo being analyzed
(C) It utilizes fluorescent DNA dyes to stain the whole embryo before blastocoele formation
(D) It amplifies highly conserved, sex-specific DNA sequences such as the SRY gene

2018

**257.** Given below are two statements regarding SCNT: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** The timing of oocyte activation is critical for the success of somatic cell nuclear transfer.
**Reason (R):** Premature activation can cause the reconstructed embryo to extrude the newly introduced somatic chromosomes.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

NODIA

2022

**258.** Which of the following best characterizes adult stem cells?
(A) They are found exclusively in early-stage pre-implantation embryos and blastocysts
(B) They are generally considered pluripotent and capable of forming all three germ layers
(C) They are located in specific niches within tissues and contribute to homeostasis and repair
(D) They divide endlessly without any molecular checkpoints or negative feedback regulation

2013

**259.** Match the reproductive hormones in List I with their primary target organ in List II.
List I: (Reproductive Hormones)
P. FSH
Q. Prostaglandin $F_2$ alpha
R. Oxytocin
S. GnRH
List II: (Primary Target Organ)
1. Corpus Luteum
2. Anterior Pituitary
3. Ovary (Follicles)
4. Uterus / Mammary Gland
(A) P-3, Q-1, R-4, S-2
(B) P-1, Q-3, R-2, S-4
(C) P-2, Q-4, R-1, S-3
(D) P-4, Q-2, R-3, S-1

2007

**260.** Consider the following statements about micro-assisted fertilization:
I. ICSI bypasses the need for the acrosome reaction.
II. PZD relies on making a slit in the zona pellucida.
III. SUZI injects sperm directly into the ooplasm.
IV. These techniques are often utilized when dealing with poor semen quality.
Which of the above statements is/are correct?

stem cells via targeted apoptotic pathways in the tissue

(D) To forcibly induce rapid terminal differentiation and specialized lineage commitment in all stem cells

2014

**276.** Arrange the following events of fertilization in the correct chronological sequence:

I. Fusion of male and female pronuclei

II. Sperm binding to the zona pellucida

III. Acrosome reaction

IV. Cortical reaction

(A) II, III, IV, I

(B) III, II, IV, I

(C) II, IV, III, I

(D) IV, II, III, I

2013

**277.** Match the genome editing techniques in List I with their primary limitation or challenge in List II.

List I: (Genome Editing Techniques)

P. Pronuclear Microinjection

Q. Homologous Recombination (traditional)

R. Retroviral Vectors

S. CRISPR-Cas9

List II: (Primary Limitation or Challenge)

1. Extremely low efficiency without engineered nucleases
2. Random integration leading to position effects
3. Potential for offF target mutations
4. Limited packaging capacity and transgene silencing

(A) P-2, Q-1, R-4, S-3

(B) P-1, Q-2, R-3, S-4

(C) P-3, Q-4, R-1, S-2

(D) P-4, Q-3, R-2, S-1

2013

**278.** Which statement correctly characterizes somatic cell co-culture systems in IVC?

(A) They use defined, serum-free synthetic chemical factors and growth supplements exclusively in the media

(B) They are only used for maturing and capacitating frozen-thawed sperm cells before standard IVF procedures

(C) They utilize a monolayer of cells to support embryo development in vitro

(D) They completely and permanently prevent the developing blastocyst from hatching out of its zona layer

2015

**279.** Given below are two statements regarding embryo splitting: one is labelled as Assertion (A) and the other is labelled as Reason (R).

**Assertion (A):** Embryo bisection is a viable technique for producing identical twin calves.

**Reason (R):** Early embryonic cells prior to lineage commitment retain the developmental plasticity to form a complete individual when separated.

(A) Both A and R are true and R is the correct explanation of A.

(B) Both A and R are true but R is not the correct explanation of A.

(C) A is true but R is false.

(D) A is false but R is true.

2005

**280.** What is the biological significance of the pre-implantation stage known as 'compaction'?

(A) It involves blastomeres flattening and forming tight junctions to establish inner and outer cell layers

(B) It marks the initial formation of the fluid-filled blastocoele cavity within the late morula stage

(C) It is the specific developmental stage where the blastocyst implants directly into the uterine wall lining

(D) It is characterized by the physical swelling, thinning of the zona, and eventual hatching of the embryo

2006

**281.** Consider the following statements about telomere dynamics in cloned animals:

I. Clones derived from adult somatic cells often inherit shortened telomeres.

II. Telomere length is heavily restored during early embryonic reprogramming, though sometimes incompletely.

III. Shortened telomeres invariably lead to severe gigantism.

IV. Telomerase is the enzyme responsible for elongating telomeres.

Which of the above statements is/are correct?

(A) I, II and IV

(B) I, III and IV

(C) II, III and IV

(D) I, II and III

2022

**282.** Which structural feature characterizes a late (expanded) blastocyst?

(A) A massive, solid ball of tightly packed and

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(A) P-2, Q-3, R-4, S-1
(B) P-1, Q-2, R-3, S-4
(C) P-3, Q-4, R-1, S-2
(D) P-4, Q-1, R-2, S-3

2010

**268.** Consider the following statements about vitrification:

I. It requires ultra-rapid cooling rates.
II. It uses high concentrations of cryoprotectants.
III. It completely avoids the formation of intra- and extra-cellular ice crystals.
IV. It is currently the preferred method for cryopreserving oocytes.
V. It strictly requires a programmable biological freezer.

Which of the above statements is/are INCORRECT?
(A) I and II
(B) V only
(C) III and IV
(D) I, II and III

2011

**269.** Which concept explains the mechanism by which CRISPR-Cas9 targets specific DNA sequences?
(A) Homologous recombination utilizing endogenous cellular DNA repair pathways
(B) Protein-protein interactions mediated by engineered zinc finger domains
(C) Random viral integration into highly transcriptionally active host genomic regions
(D) RNA-DNA base pairing guided by an sgRNA

2012

**270.** What defines the physiological phenomenon of 'capacitation'?
(A) The physical penetration and fusion of the motile sperm cell into the recipient ooplasm
(B) The rapid mitotic cell divisions of the early embryo immediately following syngamy events
(C) The biochemical changes sperm undergo in the female tract rendering them competent to fertilize
(D) The physical release of the mature fertilizable egg from the dominant ovarian follicle

2023

**271.** Identify the correct pair regarding embryo morphology and grading.
(A) Grade 2 - Severely degenerated, unviable
(B) Grade 1 - Perfect embryo, minimal fragmentation
(C) Grade 3 - Excellent candidate for freezing
(D) Grade 4 - Minor irregularities, perfectly viable

2007

**272.** Given below are two statements regarding Large Offspring Syndrome (LOS): one is labelled as Assertion (A) and the other is labelled as Reason (R).

**Assertion (A):** Large Offspring Syndrome (LOS) is frequently observed in offspring produced via SCNT.

**Reason (R):** In vitro manipulation and SCNT can cause epigenetic dysregulation of imprinted genes like $IGF_2$.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2022

**273.** Which pharmacological agent acts primarily to induce luteolysis?
(A) Prostaglandin $F_2$ alpha
(B) Follicle Stimulating Hormone (FSH)
(C) Exogenous Progesterone hormone
(D) Endogenous Ovarian Inhibin

2021

**274.** Consider the following statements regarding the production of transgenic animals via pronuclear microinjection:

I. It is technically demanding and labor-intensive.
II. It generally has a high success rate of producing functional transgenics.
III. It cannot control the exact site of transgene integration.

Which of the above statements is/are correct?
(A) I and II
(B) I and III
(C) II and III
(D) I, II and III

2002

**275.** What is the fundamental role of the 'niche' in stem cell biology?
(A) To supply a microenvironment providing signals that maintain selF-renewal and multipotency
(B) To provide a highly restrictive physical cellular barrier preventing the stem cell from migrating
(C) To selectively destroy damaged or mutated

NODIA

2016

**231.** What is the fundamental reason that embryo bisection generates identical twins?
(A) The two bisected halves contain genetically distinct cells that develop independently in the surrogate
(B) The physical bisection process induces rapid DNA replication and doubles the genetic material of cells
(C) The outer glycoprotein zona pellucida physically forces the two separated halves to grow identically
(D) The two halves are derived from the same fertilized zygote and share identical DNA

2003

**232.** Which of the following is considered the most critical factor for successful in vitro maturation of oocytes?
(A) The maintenance of gap junctions between the cumulus cells and the oocyte
(B) The physical presence of a high concentration of motile sperm cells in the tube
(C) The complete enzymatical removal of the surrounding glycoprotein zona pellucida
(D) The addition of high concentrations of a non-permeating cryoprotectant like sucrose

2002

**233.** Identify the INCORRECT statement regarding Large Offspring Syndrome (LOS).
(A) It is primarily an epigenetic phenomenon resulting from in vitro embryo manipulation and culture
(B) It solely affects the physical size of the offspring with no effect on internal organs
(C) It consistently involves the active misregulation of parent-of-origin imprinted genes like $IGF_2$
(D) It remains a highly active area of scientific concern and research in somatic cell nuclear transfer

2006

**234.** Given below are two statements regarding gene editing tools: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** CRISPR-Cas9 is highly efficient for generating gene-edited livestock compared to traditional homologous recombination.
**Reason (R):** CRISPR-Cas9 uses a guide RNA to direct site-specific double-strand breaks, greatly increasing the frequency of gene modification.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2021

**235.** What is the primary purpose of inducing superovulation in a donor animal?
(A) To maximize the number of viable embryos produced in a single estrous cycle
(B) To trigger immediate menopause and cease all subsequent follicular activity
(C) To naturally cure chronic cystic ovarian disease in highly productive cows
(D) To synchronize the estrus cycles of an entire herd of recipient animals

2001

**236.** Consider the following statements about H-Y antigen immunological assays:
I. They are based on male-specific cell surface antigens.
II. They require binding of specific antibodies to the embryo.
III. They are generally considered more accurate and reliable than PCR-based sexing.
Which of the above statements is/are correct?
(A) I and II
(B) I and III
(C) II and III
(D) I, II and III

2024

**237.** Which technique relies on the natural property of sperm to absorb and integrate foreign DNA?
(A) Pronuclear microinjection
(B) Retroviral vector integration
(C) Intracytoplasmic sperm injection
(D) Sperm-mediated gene transfer

2019

**238.** What structural modification must occur to a sperm before it can successfully undergo the acrosome reaction?
(A) It must shed its entire flagellar tail structure to reduce motility
(B) It must replicate its haploid DNA content within the maternal tract
(C) It must undergo capacitation to destabilize its plasma membrane
(D) It must bind firmly to the outer cells of the corona radiata layer

2009

**239.** Arrange the following early developmental stages in their correct chronological sequence:

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compact blastomeres lacking any internal fluid-filled cavities
(B) A distinct inner cell mass, a thinned zona pellucida, and a very large fluid-filled cavity
(C) Two distinctly visible haploid pronuclei positioned centrally within the cytoplasm before syngamy occurs
(D) The persistent presence of two polar bodies inside the perivitelline space but no distinct cell membranes

2016
**283.** Identify the INCORRECT statement regarding flow cytometry for semen sexing.
(A) The process is extremely rapid and causes absolutely zero damage or stress to the sperm
(B) X-bearing sperm emit more fluorescence than Y-bearing sperm due to higher DNA content
(C) Sperm must be correctly oriented to the laser for accurate measurement
(D) It requires the use of a vital DNA stain like Hoechst 33342

2008
**284.** Given below are two statements regarding gene pharming: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Mammary-gland specific promoters are utilized in gene pharming constructs.
**Reason (R):** They restrict the expression of the potentially bioactive therapeutic protein solely to the milk, protecting the animal's internal physiology.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2010
**285.** What represents the primary cause of polyspermy during standard in vitro fertilization?
(A) An excessively thick and impenetrable glycoprotein zona pellucida surrounding the unfertilized metaphase II oocyte
(B) The complete absence of extracellular calcium ions in the fertilization culture medium during incubation steps
(C) The use of severely decapacitated or immotile sperm prepared via sub-optimal density gradient separation protocols
(D) High sperm concentration leading to overwhelming binding before the cortical reaction can successfully complete

2014
**286.** Match the stem cell characteristics in List I with their definitions in List II.
List I: (Stem Cell Characteristics)
P. SelF-renewal
Q. Differentiation
R. Pluripotency
S. Reprogramming
List II: (Definitions)
1. The process of reverting a mature cell back to a pluripotent state
2. The ability to divide continuously while maintaining an undifferentiated state
3. The process of developing into a specialized cell type
4. The ability to give rise to any cell type of the three germ layers
(A) P-2, Q-3, R-4, S-1
(B) P-1, Q-2, R-3, S-4
(C) P-4, Q-3, R-2, S-1
(D) P-3, Q-1, R-4, S-2

2003
**287.** Consider the following statements about the role of FSH in superovulation:
I. It rescues subordinate follicles from atresia.
II. It stimulates the growth of a large cohort of follicles simultaneously.
III. It is typically administered in a single, massive dose.
IV. It causes immediate regression of the corpus luteum.
Which of the above statements is/are INCORRECT?
(A) I and II
(B) III and IV
(C) I and IV
(D) II and III

NODIA

2005
**288.** Which biological process is strictly inhibited by the administration of a progestin device (CIDR) in a cow?
(A) The synthesis of prostaglandin $F_2$ alpha
(B) The physical growth of the corpus luteum
(C) The early cleavage divisions of a fertilized embryo
(D) The preovulatory LH surge and subsequent ovulation

2007

**289.** Arrange the following tools for genome editing in order of their increasing targeting precision and modern usage:

I. Pronuclear Microinjection (Random Integration)

II. Homologous Recombination (Traditional)

III. CRISPR-Cas9

(A) I, II, III

(B) II, I, III

(C) I, III, II

(D) III, II, I

2014

**290.** What fundamentally describes the process of in vitro maturation (IVM)?

(A) Culturing a fully formed and expanded blastocyst in specialized culture media until it hatches from the zona pellucida

(B) Forcing a frozen-thawed sperm cell to undergo the physiological acrosome reaction using specialized chemical agents

(C) Culturing an oocyte until it reaches Metaphase II and extrudes the first polar body

(D) Splicing a foreign therapeutic gene sequence into the haploid genome of a single-cell fertilized zygote via microinjection

2019

**291.** Given below are two statements regarding embryo evaluation: one is labelled as Assertion (A) and the other is labelled as Reason (R).

**Assertion (A):** An embryo containing some excluded blastomeres can still successfully develop into a viable blastocyst.

**Reason (R):** Pre-implantation embryonic cells are highly plastic, allowing the remaining healthy blastomeres to compensate for cell loss.

(A) Both A and R are true and R is the correct explanation of A.

(B) Both A and R are true but R is not the correct explanation of A.

(C) A is true but R is false.

(D) A is false but R is true.

2010

**292.** Which technique utilizes a micromanipulator to inject a single sperm cell precisely into the cytoplasm of an oocyte?

(A) SCNT

(B) SUZI

(C) PZD

(D) ICSI

2022

**293.** Consider the following statements about SCNT activation:

I. Activation requires an artificial stimulus to mimic fertilization.

II. Chemical agents like ionomycin or electrical pulses are commonly used.

III. Activation triggers a massive release of intracellular calcium.

IV. Activation guarantees perfect epigenetic reprogramming.

Which of the above statements is/are INCORRECT?

(A) I and II

(B) IV only

(C) III and IV

(D) I, II and III

2009

**294.** What is the main structural barrier that sperm must overcome during natural fertilization immediately before binding to the zona pellucida?

(A) The perivitelline space

(B) The oolemma

(C) The cumulus oophorus

(D) The polar body

2011

**295.** Identify the correct statement regarding embryo freezing using slow-rate protocols.

(A) It requires the addition of a cryoprotectant in a single, massive, sudden dose to prevent osmotic shock

(B) It strictly and deliberately avoids any manual induction of extracellular ice crystals (known as seeding)

(C) It depends heavily and fundamentally on the complete and rapid formation of large intracellular ice crystals

(D) It involves a precise, gradual cooling rate (e.g., 0.3°C to 0.5°C per minute) down to roughly -30°C

2010

**296.** Match the cellular reprogramming concepts in List I with their brief descriptions in List II.

List I: (Cellular Reprogramming Concepts)

P. Genomic Imprinting

Q. Epigenetic Reprogramming

R. X-chromosome Inactivation

S. Somatic Cell Nuclear Transfer

List II: (Brief Descriptions)

1. Resetting the epigenetic marks of a differentiated nucleus to an embryonic state

2. Parent-of-origin specific differential gene

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expression
3. Technique of introducing a somatic nucleus into an enucleated oocyte
4. Dosage compensation mechanism in female mammals

(A) P-2, Q-1, R-4, S-3
(B) P-1, Q-2, R-3, S-4
(C) P-4, Q-3, R-1, S-2
(D) P-3, Q-1, R-2, S-4

2018

**297.** Given below are two statements regarding gene pharming: one is labelled as Assertion (A) and the other is labelled as Reason (R).

**Assertion (A):** Animals used for gene pharming are typically generated via SCNT utilizing transfected somatic cells.

**Reason (R):** SCNT guarantees that every resulting live offspring will carry the integrated transgene, unlike pronuclear microinjection.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2007

**298.** What defines the physiological state of pluripotency in embryonic stem cells?

(A) The highly restricted capacity of a stem cell to differentiate only into specialized red or white blood cells
(B) The capacity to differentiate into all somatic and germ lineages, but not extraembryonic tissues
(C) The absolute inability of undifferentiated cells to divide or replicate without specific native growth factors
(D) The theoretical ability to spontaneously form an entirely new, fully functional adult organism in culture dishes

2013

**299.** Consider the following statements about sexing embryos using PCR:

I. A small biopsy of trophoblast cells is taken.
II. The procedure can slightly reduce the viability of the embryo.
III. Amplification targets male-specific sequences on the Y chromosome.
IV. The presence of an amplification band confirms the embryo is definitively female.

Which of the above statements is/are INCORRECT?

(A) I and II
(B) IV only
(C) II and III
(D) I, II and III

2014

**300.** Which final step must a blastocyst complete in vivo before it can physically implant into the maternal endometrium?

(A) It must undergo the first cleavage division
(B) It must re-absorb the polar bodies
(C) It must hatch from the zona pellucida
(D) It must completely shed its inner cell mass

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mechanical control. The key instrument utilized is a micromanipulator equipped with an extremely fine microblade or needle. This device allows the operator to cleanly split the embryonic cell mass into two halves under high-magnification microscopy.

**40.** (A) Abnormal epigenetic modification of genes
Large Offspring Syndrome in cloned sheep and cattle is primarily caused by abnormal epigenetic reprogramming of the donor somatic nucleus. When somatic chromatin is transferred into an oocyte, incomplete reprogramming disrupts the methylation patterns of imprinted genes. This leads to aberrant gene expression during development, causing fetal gigantism and placental abnormalities.

**41.** (A) Both A and R are true and R is the correct explanation of A.
In somatic cell nuclear transfer, synchronizing the donor somatic cell in the $G_0$ or $G_1$ phase is critical. This specific cell cycle state ensures that the donor chromatin is compatible with the recipient oocyte cytoplasm, preventing DNA damage. This synchronization facilitates proper nuclear remodeling and genomic reprogramming, supporting normal embryonic development post-fusion.

**42.** (B) Retroviral vectors
Generating transgenic livestock via retroviral vectors exploits the natural life cycle of retroviruses, which possess the unique molecular machinery to integrate their genomic sequence into host cell nuclear DNA. Researchers package the transgene of interest inside replication-deficient viral particles and infect early embryos, leading to highly efficient integration of the foreign genetic material.

**43.** (C) IV only
Modern gene editing technologies, such as zinc finger nucleases, transcription activator-like effector nucleases, and CRISPR-Cas9, use sequence-specific targeting mechanisms to introduce genomic modifications. In contrast, traditional homologous recombination is highly inefficient and happens at extremely low rates in mammalian cells. Therefore, stating that homologous recombination is more efficient than CRISPR-Cas9 is false.

**44.** (C) It forms a limited number of cell types in a lineage
Multipotent stem cells are characterized by a restricted differentiation potential compared to pluripotent cells. They can divide and give rise to several distinct cell types, but these are strictly limited to a single tissue lineage or germ layer. Examples include hematopoietic stem cells, which produce blood cells but not neural or muscle tissues.

**45.** (D) The microenvironment regulating stem cell behavior
The stem cell niche is the specialized, highly regulated in vivo microenvironment where stem cells reside. This anatomically defined microenvironment consists of neighboring support cells, extracellular matrix components, and soluble signaling molecules. Together, these physical and chemical cues dictate whether the stem cell remains quiescent, selF-renews, or undergoes lineage-specific differentiation.

**46.** (C) Premature luteal regression
Administering a luteolytic agent, such as prostaglandin $F_2$-alpha, during the active luteal phase of the estrous cycle induces premature regression of the corpus luteum. This luteolysis causes a sudden, rapid drop in progesterone secretion. The removal of progesterone's negative feedback on the hypothalamus triggers follicular development, culminating in synchronized estrus and ovulation.

**47.** (B) ZP3
In mammalian fertilization, the initial physical docking of the sperm cell to the egg is mediated by specific glycoproteins in the zona pellucida. The ZP3 glycoprotein acts as the primary receptor that binds to complementary proteins on the sperm head membrane. This binding event is essential for initiating the subsequent acrosome reaction.

**48.** (D) Compaction - Tight junction formation
Compaction is a key transition in early pre-implantation embryo development that occurs at the late morula stage. During this process, blastomeres maximize outer cell-to-cell contact and undergo polarization. This cellular reorganization is driven by the formation of tight junctions, which seal the outer cell layer and facilitate blastocoele cavity formation during blastulation.

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**10.** (A) Both A and R are true and R is the correct explanation of A.
In vitro maturation of mammalian oocytes is highly dependent on the presence of surrounding cumulus cells. These specialized somatic cells remain physically connected to the oocyte through a network of gap junctions. Through these connections, cumulus cells transmit vital metabolic nutrients, energy substrates, and regulatory signals that drive the progression of nuclear and cytoplasmic maturation.

**11.** (A) Calcium release upon sperm-oocyte fusion
The cortical reaction is initiated immediately upon the physical fusion of the sperm with the oocyte plasma membrane. This fusion event triggers a rapid, wave-like release of intracellular calcium ions from the endoplasmic reticulum. The calcium surge drives the exocytosis of cortical granules, which modify the extracellular matrix and establish a permanent barrier to polyspermy.

**12.** (C) It eliminates disease transmission risk from serum
Defined media consist exclusively of known chemical ingredients of precise concentrations, replacing undefined biological products like serum or fluid. A primary benefit of using chemically defined formulations is the elimination of biological variability and the prevention of potential pathogen or disease transmission. This standardization improves experimental reproducibility and ensures safer embryo transfer procedures.

**13.** (D) Somatic cell nuclear transfer
Micro-assisted fertilization comprises techniques designed to assist sperm penetration, including intracytoplasmic sperm injection, partial zona dissection, and subzonal insemination. Conversely, somatic cell nuclear transfer is an asexual reproductive cloning technology rather than a fertilization technique. SCNT bypasses fertilization entirely by replacing the oocyte nucleus with a fully differentiated somatic cell nucleus.

**14.** (C) Differences in total DNA content
Sex-sorting of mammalian semen relies on the physical difference in DNA content between X and Y chromosome-bearing spermatozoa. The X chromosome is larger and contains more DNA than the Y chromosome. When sperm are stained with a DNA-binding fluorescent dye, the X-bearing sperm emit more fluorescence, allowing a flow cytometer to separate them.

**15.** (A) I, II and IV
Polymerase chain reaction sexing is highly sensitive and targets Y-chromosome specific genomic sequences. Karyotyping involves halting dividing cells during metaphase to visualize sex chromosomes directly. To extract DNA for these molecular assays, a small cellular biopsy of the blastocyst is required. Conversely, H-Y antigen assays are less reliable and have largely been replaced by PCR.

**16.** (D) To induce extracellular ice formation
During slow-freezing protocols, deliberate seeding is performed at a temperature just below the freezing point of the medium. This mechanical induction of ice crystal formation in the extracellular space creates an osmotic gradient. As a result, water is gradually drawn out of the cells, preventing the formation of lethal intracellular ice crystals during subsequent cooling.

**17.** (A) Sucrose - Permeating
Cryoprotectants are classified based on their ability to cross cellular membranes. Permeating agents, such as glycerol and dimethyl sulfoxide, pass through the lipid bilayer to protect internal organelles. Non-permeating agents, including sucrose and trehalose, remain extracellularly, establishing an osmotic gradient that dehydrates cells. Therefore, pairing sucrose as a permeating agent is scientifically incorrect.

**18.** (C) Reduction in cell number may compromise halF-embryo viability
Splitting a single embryo into two equal halves reduces the total cell number of each demicloned embryo by half. This severe reduction in cell count can compromise subsequent developmental viability, as a critical minimum number of blastomeres is required to establish the inner cell mass and trophoblast lineages, sometimes leading to pregnancy failure.

**19.** (A) II, III, I, IV
Somatic cell nuclear transfer begins with donor cell synchronization to align cell cycles, typically at the $G_0$ or $G_1$ stage. Next, the recipient oocyte is enucleated to remove maternal chromosomes. The synchronized donor cell is then fused with

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issues, modern precision gene-editing techniques do not always cause physiological abnormalities. Many genetically edited animals are completely healthy and exhibit normal growth, behavior, and reproductive capacities.

**59.** (A) It maintains the state of pluripotency
Nanog is a core homeodomain transcription factor essential for maintaining the undifferentiated, pluripotent state of embryonic stem cells. It functions in concert with Oct-4 and Sox2 to regulate gene expression networks. Together, these factors suppress genes that promote lineage-specific differentiation, thereby preserving the selF-renewal capacity of the stem cells during culture.

**60.** (C) By reprogramming somatic cells with transcription factors
Induced pluripotent stem cells are generated by reprogramming fully differentiated adult somatic cells back into an embryonic-like pluripotent state. This cellular reprogramming is achieved by introducing a specific set of transcription factors, classically including Oct-4, Sox2, Klf4, and c-Myc. These factors remodel the epigenome to restore complete selF-renewal and pluripotency.

**61.** (D) Interferon-tau
Interferon-tau is the primary pregnancy recognition signal secreted by the expanding bovine trophoblast around days fifteen to seventeen of gestation. This cytokine acts locally on the endometrium to suppress the pulsatile secretion of luteolytic prostaglandin $F_2$-alpha. Consequently, the corpus luteum is maintained, ensuring continuous progesterone production required for the pregnancy.

**62.** (A) I, II, III and V
Capacitation is a physiological maturation process occurring within the female reproductive tract, not the epididymis. It involves the removal of stabilizing seminal plasma coatings, an influx of calcium ions, and hyperactivation of sperm motility. These biochemical alterations prepare the sperm head membrane to undergo the irreversible acrosome reaction upon binding to the oocyte.

**63.** (A) The transition from maternal to embryonic genome activation
The in vitro developmental block represents a critical checkpoint where cultured mammalian embryos stop dividing and degenerate. This block typically occurs during the transition from maternal genomic control to embryonic genome activation. During this phase, the embryo must shift from utilizing maternal transcripts stored in the oocyte to transcriptionally activating its own genome.

**64.** (D) Polyvinylpyrrolidone
Non-permeating cryoprotectants are high-molecular-weight molecules that are unable to cross the plasma membrane. Polyvinylpyrrolidone, along with extracellular sugars like sucrose and trehalose, remains outside the cell during freezing. These compounds act by increasing extracellular osmolality, which draws water out of the cell, thereby reducing the risk of damaging intracellular ice crystal formation.

**65.** (A) Zygote collection, DNA injection into pronucleus, embryo transfer, screening of offspring
Generating transgenic livestock via pronuclear microinjection follows a structured pathway. First, fertilized zygotes are collected from donor animals. An engineered DNA construct is then physically microinjected directly into one of the pronuclei. The microinjected zygotes are transferred into synchronized recipient surrogates, and the resulting offspring are screened for successful integration.

**66.** (A) Both A and R are true and R is the correct explanation of A.
During somatic cell nuclear transfer, the physical processes of enucleation and cell fusion do not trigger the normal calcium oscillations initiated by a fertilizing sperm. Consequently, artificial chemical or electrical activation of the reconstructed oocyte is mandatory. This activation induces calcium releases that mimic fertilization, restarting the cell cycle to drive embryonic development.

**67.** (A) Telomere shortening
Telomeres are repetitive DNA sequences located at the ends of linear chromosomes that protect genomic integrity. When adult somatic cells are used as nuclear donors in cloning, their telomeres may already be shortened due to age. If reprogramming fails to restore telomere length, the cloned offspring may suffer from premature cellular senescence and aging.

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follicle-stimulating and luteinizing hormones to rise, prompting follicular growth, estrogen secretion, and the onset of synchronized estrus.

**30.** (C) Estrus synchronization
The Controlled Internal Drug Release device is an intravaginal insert containing progesterone, widely used in cattle estrus synchronization protocols. It continuously releases progesterone to mimic a functional corpus luteum, preventing premature estrus and ovulation. Upon removal of the device, progesterone levels fall rapidly, inducing synchronized follicular development and estrus across animals.

**31.** (B) Symmetrical, spherical mass with minimal extruded cells
Under the International Embryo Technology Society guidelines, a Grade 1 embryo is defined by high morphological symmetry and uniformity. The blastomeres should be regular in size, color, and density, forming a cohesive spherical mass. The presence of extruded cells, blastomere fragmentation, or irregularities in the zona pellucida must be minimal or absent entirely.

**32.** (A) Cleavage medium followed by blastocyst medium
In vitro culture of mammalian embryos requires sequential media systems to support changing metabolic requirements. Cleavage-stage embryos have low glucose requirements and rely primarily on pyruvate and lactate for energy. Thus, they are initially cultured in cleavage medium, which is subsequently replaced by blastocyst medium containing higher concentrations of glucose and amino acids.

**33.** (A) I and II
Somatic cell co-culture systems were historically developed to overcome the developmental block in vitro. Helper cells, such as oviductal epithelial cells, improve development by secreting beneficial embryotrophic factors, including growth factors and substrates, while removing toxic compounds from the medium. Modern chemically defined media have largely replaced co-culture but have not been replaced by serum.

**34.** (C) ICSI
Intracytoplasmic sperm injection is a micro-assisted fertilization technique performed under a high-magnification micromanipulator. It involves capturing a single viable spermatozoon, immobilizing it, and directly injecting it through the outer zona pellucida and oolemma into the ooplasm of a metaphase II oocyte. This bypasses all natural sperm penetration steps.

**35.** (A) The X sperm contains approximately 3.8% more DNA
In bovines, the X chromosome is substantially larger than the Y chromosome, leading to a measurable difference in genomic DNA content. Sperm containing the X chromosome possess approximately 3.8% more total DNA than those carrying the Y chromosome. This difference is exploited by flow cytometry to separate the two populations.

**36.** (D) PCR-based amplification
Polymerase chain reaction is an extremely sensitive molecular diagnostic tool used for embryo sexing. It specifically amplifies male-associated DNA sequences, such as SRY or other Y-chromosome specific markers, from a tiny embryo biopsy. This technique identifies the sex of the embryo with high accuracy without requiring the cultivation of live cells.

**37.** (B) P-2, Q-3, R-4, S-1
Cryoprotective agents are essential for preserving cells at low temperatures. Permeating agents cross the plasma membrane to prevent intracellular ice formation, whereas non-permeating agents remain extracellular, promoting cellular dehydration through osmosis. Cryoprotectant toxicity increases with both concentration and temperature. Vitrification represents ultra-rapid physical cooling that leads to ice-free, glass-like solidification.

**38.** (C) It relies on extracellular ice to dehydrate cells
Vitrification is an advanced cryopreservation technique that completely avoids the formation of any intracellular or extracellular ice crystals. It achieves a glass-like solid state by combining highly concentrated cryoprotectant solutions with extremely fast physical cooling rates. Therefore, stating that vitrification relies on extracellular ice formation to dehydrate cells is scientifically incorrect.

**39.** (D) Micromanipulator with blade
Mechanical bisection of embryos, typically performed at the morula or blastocyst stage to generate identical twins, requires precise

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the enucleated oocyte using electrofusion. Finally, artificial activation of the reconstituted zygote initiates embryonic development.

**20.** (D) Large Offspring Syndrome
Incomplete or incorrect epigenetic reprogramming of the donor somatic nucleus during cloning is the primary driver of developmental abnormalities. The most prominent phenotype in cloned ruminants is Large Offspring Syndrome. This condition is characterized by placental overgrowth, prolonged gestation, fetal gigantism, and organomegaly, which stem from abnormal expression patterns of imprinted genes.

**21.** (B) Higher targeted double-strand break efficiency
The CRISPR-Cas9 system represents a major advancement in genome editing due to its high efficiency in generating targeted double-strand breaks. Unlike traditional homologous recombination, which is highly inefficient and occurs at extremely low frequencies, Cas9-mediated cleavage is guided by a specific RNA sequence, dramatically improving targeting success rates in livestock embryos.

**22.** (B) III only
Gene pharming uses transgenic bioreactor animals to produce valuable human therapeutic proteins, primarily targeting secretion into milk rather than blood. This specificity is achieved by linking the transgene of interest to mammary-gland specific promoters, such as beta-lactoglobulin or casein. Thus, stating that bioreactor animals produce recombinant proteins in their blood only is incorrect.

**23.** (B) P-1, Q-2, R-3, S-4
Stem cells are classified based on their differentiation potential. Totipotent cells can form all embryonic and extraembryonic tissues. Pluripotent cells can differentiate into all three embryonic germ layers but cannot form placental structures. Multipotent cells are restricted to forming multiple cell types within a specific lineage, while unipotent cells can generate only one specific mature cell type.

**24.** (A) CD45
Embryonic stem cell characterization relies on specific pluripotency markers, including the transcription factors Oct-4 and Nanog, along with stage-specific embryonic antigens on the cell surface. Conversely, CD45 is a transmembrane protein tyrosine phosphatase expressed specifically on immune cells and hematopoietic lineages, making it unsuitable as a marker for undifferentiated embryonic stem cells.

**25.** (B) Generating patient-specific cells for therapy
The primary clinical utility of induced pluripotent stem cells in veterinary medicine is the generation of patient-specific cells for regenerative therapies. By reprogramming adult somatic cells back to a pluripotent state, researchers can produce autologous differentiated cells that can be transplanted back into the donor animal, eliminating the risk of immune rejection.

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**26.** (B) Estrogen
During the follicular phase of the estrous cycle, growing follicles produce increasing amounts of estrogen. Once blood concentrations of estrogen reach a threshold, they exert positive feedback on the hypothalamus and anterior pituitary. This feedback triggers the sudden, massive secretion of luteinizing hormone, which is responsible for inducing follicle rupture and ovulation.

**27.** (A) It is an irreversible exocytotic process required for zona pellucida penetration
The acrosome reaction is a highly regulated, irreversible exocytotic event that occurs when a capacitated sperm binds to receptors on the zona pellucida. This reaction releases specialized hydrolytic enzymes, such as acrosin, which digest the extracellular matrix. This physical and chemical process is absolutely required for the sperm to penetrate the egg.

**28.** (C) Formation of the morula
The cleavage stage of pre-implantation embryonic development is characterized by rapid mitotic divisions of the zygote without any net increase in cytoplasmic volume. This developmental phase officially concludes with the formation of the morula, which is a solid spherical ball of blastomeres. Subsequent development transitions into compaction and blastulation.

**29.** (A) Both A and R are true and R is the correct explanation of A.
Prostaglandin $F_2$-alpha is a potent luteolytic agent administered to regress the mature corpus luteum in cyclic livestock. Luteal regression leads to a rapid decline in circulating progesterone levels. This removal of negative feedback allows

**49.** (A) IV, II, III, I
The superovulation and embryo transfer protocol begins with multiple injections of FSH to stimulate follicular growth. Next, prostaglandin is administered to induce luteolysis, bringing the donor animal into estrus. Artificial insemination is then performed to fertilize the multiple ovulated eggs. Finally, non-surgical embryo flushing is executed seven days later to harvest developed blastocysts.

**50.** (C) To remove infectious agents on the zona pellucida
Washing recovered embryos multiple times in sterile medium is a strict biosafety requirement defined by the International Embryo Technology Society. The primary purpose is to physically detach and remove any pathogens, bacteria, or viruses that may adhere to the outer surface of the zona pellucida. This prevents disease transmission to the recipient animal.

**51.** (B) I, III and IV
Grading of cumulus-oocyte complexes determines their developmental competence for in vitro maturation. Grade A complexes possess a compact, multi-layered cumulus investment, making them ideal for culture. Denuded oocytes lack cumulus cells entirely and exhibit low maturation rates. Grade B oocytes have partial cumulus coverage, whereas expanded, dark cumulus layers characterize degenerating or atretic complexes.

**52.** (D) Cortical granule release altering the zona
The primary slow block to polyspermy in mammalian fertilization is mediated by the cortical reaction. Upon sperm-oocyte fusion, intracellular calcium levels rise, triggering the exocytosis of cortical granules into the perivitelline space. These granules release hydrolytic enzymes that modify and harden the glycoproteins of the zona pellucida, permanently preventing further sperm entry.

**53.** (A) P-2, Q-1, R-3, S-4
Assisted reproductive technologies serve distinct clinical and research purposes. In vitro fertilization generates viable embryos from collected male and female gametes. Intracytoplasmic sperm injection treats severe male infertility by introducing a single sperm into the egg. Somatic cell nuclear transfer produces cloned animals, while flow cytometry separates sperm cells based on sex chromosome differences.

**54.** (C) Limited gene packaging capacity
Although retroviral vectors are highly efficient at integrating foreign DNA into the host genome, they have a major biological limitation regarding genetic packaging size. The viral capsid can only accommodate relatively small genetic constructs, typically under eight kilobases. This restriction prevents the delivery of large mammalian genes, complex regulatory sequences, or multiple transgene cassettes.

**55.** (A) Both A and R are true and R is the correct explanation of A.
Homologous recombination is an established molecular biology technique used to disrupt or insert specific genes. It relies on introducing a targeting vector constructed with flanking DNA sequences homologous to the genomic target site. These homologous regions align and undergo crossing over with the host chromosomes, replacing the endogenous gene with the engineered sequence.

**56.** (A) Using transgenic animals to produce drugs
Gene pharming refers to the use of genetically modified or transgenic livestock to produce valuable pharmaceuticals, antibodies, or industrial proteins. The transgene of interest is typically coupled with a tissue-specific promoter, allowing the therapeutic protein to be synthesized and secreted in large quantities into animal biofluids, most commonly female milk.

**57.** (B) Beta-lactoglobulin promoter
To ensure that a recombinant protein is secreted specifically into the milk of a transgenic bioreactor animal, researchers utilize mammary-gland specific promoters. The beta-lactoglobulin promoter is a highly efficient regulatory sequence derived from milk protein genes. When linked to the gene of interest, it restricts transcriptional activity exclusively to lactating mammary epithelial cells.

**58.** (B) It always results in severe physiological abnormalities
Integrating economically important genes in livestock aims to improve agricultural traits such as wool quality, muscle development, and disease resistance. While early transgenic experiments occasionally caused developmental

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# ANSWERS

**1.** (A) Follicle-stimulating hormone
Follicle-stimulating hormone, secreted by the anterior pituitary gland, is the primary endocrine signal that initiates the growth and recruitment of a new wave of ovarian follicles in livestock. This hormone supports the proliferation of granulosa cells and estrogen production, which are essential for early follicular development before dominance is established.

**2.** (C) It secretes high levels of estrogen
In bovine reproductive physiology, the corpus luteum is a temporary endocrine structure formed after ovulation. Its primary functional role is the production and secretion of high levels of progesterone, which is critical for maintaining pregnancy. Conversely, it does not secrete high levels of estrogen, as estrogen is mainly produced by developing follicles.

**3.** (B) Capacitation, acrosome reaction, cortical reaction
Fertilization in mammals begins with sperm capacitation within the female reproductive tract, which confers fertilizing ability. This is followed by the acrosome reaction, allowing the sperm to penetrate the outer extracellular matrix. Finally, the cortical reaction occurs upon sperm-oocyte fusion, releasing enzymes that modify the zona pellucida to prevent polyspermy.

**4.** (A) P-4, Q-3, R-2, S-1
Early mammalian development involves several distinct physiological stages. Mitotic divisions without cytoplasmic growth define cleavage. Compaction occurs when blastomeres maximize cell-to-cell contact through tight junctions. Blastulation is characterized by the formation of a fluid-filled blastocoele cavity. Finally, hatching represents the process where the expanding blastocyst physically escapes from the protective outer zona pellucida.

**5.** (B) Sperm maturation within the female reproductive tract
Capacitation is a crucial physiological maturation process that sperm must undergo inside the female reproductive tract to gain fertilizing capacity. This activation involves the removal of stabilizing seminal plasma proteins, changes in membrane lipid composition, and an influx of calcium ions, which collectively enable hyperactivated motility and prepare the sperm for the acrosome reaction.

**6.** (C) Progestin device followed by prostaglandin $F_2$ alpha
Estrus synchronization in cyclic cows is effectively achieved using a controlled internal drug release progestin device combined with prostaglandin administration. The progestin acts as an artificial corpus luteum, suppressing ovulation and estrus. Administering prostaglandin at the end of the treatment regresses any natural luteal tissue, ensuring synchronized estrus across the herd.

**7.** (A) I and II
Equine chorionic gonadotropin has a significantly longer biological halF-life than follicle-stimulating hormone in cattle, which allows it to be administered in a single dose. Conversely, follicle-stimulating hormone must be injected multiple times over several days because it is cleared rapidly from circulation. Human chorionic gonadotropin is used to induce ovulation, not luteolysis.

**8.** (A) It aligns uterine and embryonic development stages
Achieving precise estrus synchrony between the donor and recipient is essential for successful embryo transfer. This alignment ensures that the uterine microenvironment of the recipient is at the exact physiological stage required to support the developmental needs of the transferred embryo. Mismatches can lead to maternal-embryonic asynchrony, resulting in failed implantation.

**9.** (B) Color of the zona pellucida
According to the International Embryo Technology Society guidelines, embryo grading relies on key morphological features of the embryonic mass. These include the degree of cellular fragmentation, the uniformity and symmetry of blastomeres, and the stage of development relative to the age. The color of the outer zona pellucida is not a biologically relevant grading parameter.

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**68.** (D) Perivitelline space
Subzonal insemination is an early micro-assisted fertilization technique developed to bypass barriers in the outer vestments of the egg. Under microscopic guidance, a microinjection pipette is passed through the outer zona pellucida, and sperm cells are deposited directly into the perivitelline space, which is the area located between the zona and the oolemma.

**69.** (A) P-3, Q-4, R-2, S-1
Superovulation and luteolytic hormones originate from distinct anatomical tissues. Equine chorionic gonadotropin is produced by equine endometrial cups during horse pregnancy. Human chorionic gonadotropin is secreted by the human syncytiotrophoblast. Follicle-stimulating hormone is synthesized and released by the anterior pituitary gland, while prostaglandin $F_2$-alpha is produced by the endometrium.

**70.** (C) It provides the exact same nutrients from Day 1 to Day 7
Sequential media systems are designed to support the dynamic, changing metabolic needs of the developing embryo from fertilization to the blastocyst stage. These formulations do not provide the exact same nutrients throughout culture. Instead, early media contain lower glucose and higher lactate concentrations, whereas later media increase glucose and amino acid levels.

**71.** (D) To induce sperm capacitation
Bovine spermatozoa must undergo capacitation to fertilize an oocyte. In vitro, this physiological process is artificially induced by adding glycosaminoglycans, most commonly heparin, to the fertilization medium. Heparin binds to seminal plasma proteins on the sperm surface, promoting cholesterol efflux and intracellular calcium changes necessary to prepare the sperm for the acrosome reaction.

**72.** (A) Morula or blastocyst
For non-surgical embryo transfer in cattle, embryos are typically harvested and transferred on day seven or eight post-estrus. At this stage, the embryos have reached the compact morula or blastocyst stage. These stages are preferred because they align with the uterine environment of the recipient, maximizing pregnancy rates compared to early cleavage-stage embryos.

**73.** (B) II only
The H-Y antigen assay is an immunological approach for sexing embryos that utilizes antibodies targeting male-specific surface antigens. Unlike invasive karyotyping or PCR assays, this method is non-destructive, as the antibody binding does not compromise embryo viability. Thus, stating that the H-Y antigen assay requires the complete destruction of the embryo is incorrect.

**74.** (B) It eliminates programmable freezers
Vitrification is an ultra-rapid cooling technique that solidifies liquid into a glass-like state without ice crystal formation. A major practical advantage of vitrification over traditional slow-freezing is that it eliminates the need for expensive, specialized programmable freezers. The embryos can be placed directly into liquid nitrogen, making the procedure faster and more field-friendly.

**75.** (A) It secretes inhibin to suppress FSH
During a follicular wave, a single follicle is selected to become dominant. This dominant follicle secretes high levels of estradiol and inhibin, which suppress circulating follicle-stimulating hormone levels. By reducing FSH, the dominant follicle effectively starves smaller subordinate follicles of necessary gonadotropic support, causing them to undergo atresia while it continues to grow.

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**76.** (C) They release enzymes to harden the zona
Cortical granules are specialized membrane-bound organelles located beneath the oocyte plasma membrane. Upon sperm penetration, a calcium wave triggers their exocytosis. The granules release hydrolytic enzymes into the perivitelline space, which cleave sperm receptors and cross-link the zona pellucida proteins. This structural hardening of the zona prevents additional sperm from fertilizing the egg.

**77.** (D) Cas9 endonuclease
The CRISPR system achieves precise, targeted gene disruption through the activity of the Cas9 endonuclease enzyme. Cas9 is guided to a specific genomic locus by a custom single-guide RNA sequence. Once positioned, the enzyme introduces a double-strand break in the host DNA, which is repaired by error-prone cellular pathways, leading to gene inactivation.

swelling and membrane rupture, combined with chemical toxicity from prolonged exposure.

**137.** (D) I, II and III
Monozygotic twins represent genetically identical siblings. They can occur naturally when the inner cell mass of a blastocyst splits during development. They can also be produced artificially through mechanical embryo bisection under a microscope. Because they originate from a single fertilized egg, monozygotic twins always share the exact same genetic sex.

**138.** (B) Flow cytometry using Hoechst dye
Semen sexing is achieved by staining spermatozoa with a DNA-specific fluorescent dye, typically Hoechst 33342. Because X-chromosome bearing sperm contain slightly more total genomic DNA than Y-bearing sperm, they bind more dye. When passed through a flow cytometer, the X-sperm emit a stronger fluorescent signal, allowing precise separation.

**139.** (A) P-4, Q-3, R-1, S-2
Early bovine embryonic development follows a predictable chronological timeline post-estrus. The initial two-cell cleavage stage occurs early on days one to two. The embryo develops into a morula by days five to six. It then transitions into a blastocyst around days seven to eight, before finally hatching on days nine to ten.

**140.** (C) Unusually long animal lifespan
Large Offspring Syndrome is a complex developmental disorder in cloned ruminants, characterized by high birth weights, placental abnormalities, and developmental defects. This condition is associated with high neonatal mortality and multi-organ dysfunction. Affected clones do not exhibit unusually long lifespans; instead, their life expectancy is often significantly reduced due to these systemic abnormalities.

**141.** (A) Both A and R are true and R is the correct explanation of A.
Random genomic integration of transgenes via pronuclear microinjection often results in highly variable transgene expression levels. This variability is driven by position effect variegation. The transcription of the integrated transgene is heavily influenced by the chromatin state of the surrounding host DNA, with integration into transcriptionally silent heterochromatin silencing expression.

**142.** (C) Transcription factor maintaining pluripotency
Oct-4 is a critical homeodomain transcription factor encoded by the Pou5f1 gene, expressed specifically in undifferentiated stem cells. It acts as a master regulator of the pluripotency network, working alongside other factors to maintain selF-renewal. Downregulation of Oct-4 triggers rapid cellular differentiation, while its expression preserves the developmental potential of the cells.

**143.** (B) Somatic cell cloning is highly efficient
Somatic cell nuclear transfer faces major biological challenges, making the overall cloning process highly inefficient. Typically, fewer than five percent of reconstructed cloned embryos develop into viable, healthy offspring. This low efficiency is caused by failures in epigenetic remodeling, aberrant DNA methylation, chromatin structure defects, and incomplete reprogramming of the donor nucleus.

**144.** (D) Inducing extracellular ice formation
In cryobiology, seeding is the deliberate induction of ice crystal formation in the extracellular medium during slow-freezing. This is achieved by briefly touching the side of the straw with a cold instrument. Seeding initiates controlled extracellular crystallization, establishing an osmotic gradient that gradually dehydrates the cells and prevents lethal intracellular ice formation.

**145.** (C) IV only
PCR-based embryo sexing is an extremely rapid molecular diagnostic technique. It requires taking a small biopsy of cells, typically from the trophectoderm, and extracting their DNA. PCR is then used to amplify highly conserved, Y-chromosome specific target sequences, such as the SRY gene. This entire process is highly accurate and is completed within several hours, not months, making statement IV incorrect.

**146.** (A) II, I, IV, III
An in vitro fertilization protocol follows a strict chronological sequence. First, immature oocytes are harvested through follicular aspiration of antral follicles. The recovered cumulus-oocyte complexes are graded under microscopy to select high-quality gametes. These complexes undergo in vitro maturation, and are finally co-incubated

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**88.** (A) It guarantees monospermic fertilization
Partial zona dissection is a micro-assisted fertilization technique that involves creating a mechanical opening in the outer zona pellucida of the oocyte. This opening facilitates sperm access to the perivitelline space, helping bypass a hardened zona. However, creating this opening increases the risk of multiple sperm entry and does not guarantee monospermic fertilization.

**89.** (B) The stem cell niche hypothesis
The maintenance of healthy stem cell populations in adult organisms is explained by the stem cell niche hypothesis. This concept states that stem cells reside in specialized, anatomically defined microenvironments that provide structural support and crucial regulatory signals. These signals control the balance between selF-renewal, quiescence, and lineage-specific differentiation.

**90.** (A) Both A and R are true and R is the correct explanation of A.
Sex-sorting of semen using flow cytometry relies on differences in DNA content between sperm. X-chromosome bearing sperm have a larger chromosome and contain more DNA than Y-chromosome bearing sperm. When stained with a DNA-specific dye, X-sperm absorb more dye and emit stronger fluorescence, enabling the cytometer to sort them accurately.

**91.** (A) Plus or minus 24 hours
Successful bovine embryo transfer requires precise synchrony between the estrous cycles of the donor and recipient animals. The ideal window of synchrony is within plus or minus twenty-four hours. This close alignment ensures that the uterine endometrium of the recipient is receptive to the development stage of the transferred day-seven blastocyst.

**92.** (B) II and IV
In vitro culture of mammalian embryos is highly dependent on media composition and environmental conditions. Semi-defined media often incorporate bovine serum albumin to provide essential protein support. Furthermore, reducing oxygen tension to five percent significantly improves embryo development and blastocyst quality compared to atmospheric oxygen levels by reducing oxidative stress.

**93.** (D) Sperm binding exogenous DNA and carrying it to the egg
Sperm-mediated gene transfer is a method used to produce transgenic animals. This technique relies on the natural ability of mature spermatozoa to bind exogenous DNA molecules present in their environment. During fertilization, the sperm acts as a biological vector, carrying the foreign genetic material directly into the oocyte, leading to genomic integration.

**94.** (C) High progesterone, low estrogen
The luteal phase of the estrous cycle is dominated by the presence of a functional corpus luteum. This endocrine structure secretes high levels of progesterone, which prepares the uterus for pregnancy and suppresses estrus behavior. Because large follicular development is inhibited by progesterone, estrogen levels remain low during this phase.

**95.** (B) Blastocoele cavity formation
The transition from the morula to the blastocyst stage is defined by the formation of the blastocoele. As outer blastomeres establish tight junctions during compaction, sodium pumps begin accumulating fluid inside the intercellular spaces. This fluid coalesces to form a single large, fluid-filled cavity, organizing the embryo into distinct cell lineages.

**96.** (D) Long halF-life causes cystic ovaries
Equine chorionic gonadotropin has a very long biological halF-life in cattle due to its high sialic acid content. While this allows for a single injection, its prolonged circulation continues to stimulate the ovaries long after ovulation. This persistent stimulation can lead to abnormal follicular development, ovarian cysts, and poor-quality embryos.

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**97.** (A) An embryo with severe fragmentation is graded as poor or degenerate
Embryo evaluation and grading rely on key morphological features observed under stereomicroscopy. An embryo exhibiting severe blastomere fragmentation, cell extrusion, or irregular division patterns is classified as poor or degenerate. These abnormalities indicate compromised developmental potential, making such embryos highly unsuitable for cryopreservation or transfer into recipient surrogates.

**117.** (B) Embryos are deposited into the uterine horn ipsilateral to the corpus luteum
During embryo transfer in cattle, the embryo must be deposited into the uterine horn ipsilateral to the ovary containing the corpus luteum. This local positioning ensures the embryo benefits from high local concentrations of progesterone and maternal recognition signals. Depositing the embryo in the contralateral horn significantly reduces pregnancy rates.

**118.** (C) To scavenge radicals and secrete growth factors
Before the development of modern chemically defined media, somatic cell co-culture systems were widely used to support in vitro embryo development. Support cells, such as oviductal epithelial cells, improve the microenvironment by actively scavenging harmful free radicals and metabolic waste products. They also secrete essential embryotrophic factors and growth factors that enhance embryo viability.

**119.** (D) SCNT followed by blastocyst isolation
Somatic cell nuclear transfer followed by inner cell mass isolation from the resulting blastocyst enables the generation of patient-specific embryonic stem cells. Because the nuclear DNA of the clone is derived entirely from the adult patient's somatic donor cell, these stem cells are genetically matched to the patient, preventing immunological rejection upon transplantation.

**120.** (A) Both A and R are true and R is the correct explanation of A.
Rapid thawing is mandatory for embryos frozen using slow-freezing protocols. During slow-freezing, small, non-lethal extracellular ice crystals form. If thawing is too slow, these tiny crystals can recrystallize, merging into larger, sharp ice crystals that physically rupture cell membranes. Rapid warming prevents this recrystallization, ensuring high post-thaw survival rates.

**121.** (B) Aiding fertilization through a hardened zona
Subzonal insemination is a micro-assisted fertilization technique developed to bypass physical blocks on the egg surface. In cases where the outer zona pellucida is abnormally hard or resistant to sperm penetration, a micro-pipette is used to deposit several sperm cells directly into the perivitelline space, facilitating direct contact with the oolemma.

**122.** (B) I, II and III
Stage-specific embryonic antigens are cell surface glycolipids widely used as markers to characterize stem cells. Their expression profiles are highly dynamic, changing predictably as pluripotent embryonic stem cells differentiate into more restricted lineages. These cell surface molecules are not located in the mitochondria, making their characterization as mitochondrial markers incorrect.

**123.** (A) Embryo transit time from oviduct to uterus
The optimal timing for non-surgical embryo flushing in cattle is day seven post-estrus. This timing is dictated by the physiological transit rate of the developing embryos from the oviducts into the uterine horns. Performing the flush on day seven ensures that the embryos have entered the uterus but have not yet hatched.

**124.** (C) An animal with a disrupted or inactivated gene
A knockout animal is a genetically engineered model in which a specific endogenous gene has been intentionally disrupted, mutated, or completely inactivated. This is achieved through targeted gene targeting or genome editing techniques. Disrupting the gene allows researchers to study its functional role in development, health, physiology, and disease pathogenesis.

**125.** (B) II, I, III
An expression vector designed for gene pharming must be assembled in a precise transcriptional sequence from 5' to 3'. It begins with a mammary-specific promoter to drive tissue-specific transcription. This is followed by the gene of interest encoding the therapeutic protein, and concludes with a polyadenylation signal to stabilize the mRNA transcript.

**126.** (C) Fragmentation and chromatin condensation
Apoptosis is a form of programmed cell death that plays a critical role in early embryo development. It is characterized by highly conserved structural changes, including nuclear chromatin condensation and the fragmentation of the cell into membrane-bound apoptotic bodies. This controlled process removes damaged or abnormal cells without causing inflammation or lysing tissue.

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genes. It is not caused by viral infections during pregnancy.

**108.** (C) To induce targeted genomic double-strand breaks
Transcription activator-like effector nucleases are engineered restriction enzymes designed to facilitate precise genome editing. They consist of modular DNA-binding domains fused to a FokI endonuclease domain. Upon binding to their target sequence, they induce a site-specific double-strand break in the host DNA, which activates cellular repair pathways to introduce gene modifications.

**109.** (A) Proestrus, Estrus, Metestrus, Diestrus
The mammalian estrous cycle is divided into four distinct sequential phases. It begins with proestrus, characterized by follicular growth and rising estrogen. This is followed by estrus, the period of sexual receptivity and ovulation. Next, metestrus is marked by the formation of the corpus luteum, which matures fully during the diestrus phase.

**110.** (C) They serve as surface markers for cell isolation
Cluster of differentiation markers are cell surface proteins expressed on different cell lineages. In adult stem cell biology, specific combinations of CD markers serve as critical molecular signatures. Clinically, these surface markers are utilized to isolate, purify, and characterize specific stem cell populations, such as hematopoietic or mesenchymal stem cells, from heterogeneous tissues.

**111.** (C) A is true but R is false.
The Ovsynch protocol utilizes injections of both gonadotropin-releasing hormone and prostaglandin $F_2$-alpha to synchronize ovulation. Prostaglandin alone cannot synchronize ovulation in anestrus cows because its action depends strictly on the presence of a functional, mature corpus luteum. In anestrus animals, there is no active luteal tissue to regress, making prostaglandin ineffective on its own.

**112.** (D) Enzymatic degradation paired with blastocyst expansion
Hatching is the physical escape of the blastocyst from the outer zona pellucida. This biological process is driven by two cooperative mechanisms: the secretion of proteolytic enzymes, such as trypsin-like proteases from the trophoblast, which locally digest the zona, and the rhythmic expansion and contraction cycles of the blastocoele that structurally weaken the matrix.

**113.** (B) II and IV
In vitro fertilization protocols require precise timing and preparation of gametes. The co-incubation of sperm and oocytes typically lasts for eight to eighteen hours, not twenty-four to forty-eight hours. Furthermore, cumulus-oocyte complexes are not stripped of their cumulus cells before co-incubation, as these cells are essential for supporting sperm penetration and fertilization.

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**114.** (C) A linear DNA construct with gene and promoter
Traditional pronuclear microinjection protocols utilize a purified, linear double-stranded DNA construct. This construct contains the promoter sequence linked directly to the transgene of interest and a polyadenylation signal, with all plasmid backbone sequences removed. Linearization of the transgene improves integration efficiency into the host genome during early cleavage divisions.

**115.** (A) Changing gene expression without altering DNA sequence
Epigenetic reprogramming refers to the cellular process of altering gene expression profiles without changing the primary genomic DNA sequence. This is accomplished through reversible modifications of the chromatin structure, such as DNA methylation and histone acetylation. These chemical modifications act as functional switches, turning specific genes on or off during embryonic development.

**116.** (A) P-3, Q-4, R-2, S-1
Reproductive hormones regulate distinct physiological functions. Gonadotropin-releasing hormone stimulates the release of FSH and LH from the anterior pituitary. Progesterone maintains pregnancy and suppresses estrous behavior. Oxytocin induces milk let-down and uterine contractions during parturition. Inhibin acts via a negative feedback loop to suppress the synthesis and release of follicle-stimulating hormone.

**156.** (B) Vitrification employs ultra-rapid cooling to achieve an ice-free glass-like state
Vitrification utilizes ultra-rapid cooling rates combined with high concentrations of cryoprotectants to instantly solidify the liquid phase without forming any ice crystals. This process transitions the solution directly into an amorphous, glass-like state. In contrast, slow-freezing protocols rely on gradual cooling rates and lower cryoprotectant concentrations, which inevitably allow ice crystals to grow extracellularly.

**157.** (A) P-3, Q-1, R-2, S-4
Estrogen is synthesized and secreted by the granulosa cells of developing ovarian follicles. Follicle-stimulating hormone is produced and released by gonadotroph cells in the anterior pituitary gland. Progesterone is synthesized by the corpus luteum to maintain pregnancy. Interferon-tau is a critical signal secreted by the embryonic trophoblast to prevent maternal luteolysis in ruminants.

**158.** (D) Denuded oocytes mature much faster and better in vitro than intact COCs
Cumulus cells play an essential role in supporting the growth, cytoplasmic maturation, and developmental competence of the oocyte. They transmit vital metabolic substrates, nucleotides, and signaling molecules through specialized gap junctional networks. Removing these cells prior to maturation compromises the oocyte's environment, so denuded oocytes exhibit significantly lower maturation rates and poor developmental quality.

**159.** (B) Extremely high sperm concentrations in the co-incubation drop
Under natural conditions, the number of sperm reaching the fertilization site is highly restricted. In vitro fertilization systems often exceed these physiological numbers. Exposing oocytes to excessive concentrations of sperm in the droplet allows multiple sperm to penetrate the zona pellucida simultaneously before the oocyte can complete the cortical reaction and establish an effective block.

**160.** (A) Both A and R are true and R is the correct explanation of A.
CRISPR-Cas9 has revolutionized livestock genome editing due to its remarkable ease of design and high efficiency compared to zinc finger nucleases. The target specificity of Cas9 is determined by simple Watson-Crick base pairing of a guide RNA sequence. This eliminates the necessity for complex, expensive, and time-consuming custom protein engineering required by older nucleases.

**161.** (A) Electrofusion
Electrofusion is a physical method that applies a brief, high-voltage electrical pulse to destabilize and transiently open pores in the adjacent cell membranes of the donor somatic cell and the enucleated oocyte. This membrane disruption induces cell fusion, successfully transferring the donor nucleus into the host cytoplasm to reconstruct a cloned embryo during nuclear transfer procedures.

**162.** (C) III only
Transgenesis is a powerful tool to introduce desirable traits like disease resistance into livestock, often using retroviral vectors to integrate foreign DNA. However, expression of the integrated transgene in the offspring of a founder animal is not guaranteed to be identical. Variable transgene expression occurs due to mosaicism, position effects, gene silencing, and epigenetic modifications.

**163.** (A) The ability to divide and produce all differentiated cells, including extraembryonic tissues
Totipotency is the ultimate developmental state where a single cell can divide and differentiate into every specialized cell type of the complete organism. This unique potential includes forming both embryonic lineages and essential extraembryonic structures, such as the placenta. Early blastomeres from the zygote up to the early cleavage stages possess this complete developmental capacity.

**164.** (D) Oct-4
Octamer-binding transcription factor 4 is a critical nuclear protein encoded by the Pou5f1 gene. It is widely recognized as a master regulator of pluripotency in embryonic stem cells. This transcription factor works in network with other proteins to repress differentiation-specific genes, thereby preserving the undifferentiated, selF-renewing state of cells within the inner cell mass of blastocysts.

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**127.** (B) Capacitation - Occurs in the epididymis
Gamete maturation and fertilization involve highly coordinated processes. Syngamy is the fusion of pronuclei, the cortical reaction blocks polyspermy, and the acrosome reaction releases enzymes to digest the zona. Conversely, capacitation does not occur in the epididymis; it is an activation process that takes place inside the female reproductive tract post-ejaculation.

**128.** (D) To supply proteins, growth factors, and vitamins
Fetal bovine serum is widely added to in vitro culture media to support embryo development. Serum acts as a rich source of essential macromolecules, including transport proteins, attachment factors, vitamins, and physiological growth factors. These components promote cell proliferation, stabilize cell membranes, and help protect the developing embryos from chemical fluctuations in vitro.

**129.** (A) P-3, Q-1, R-2, S-4
Micro-assisted fertilization and micromanipulation techniques utilize distinct mechanical operations. ICSI involves the direct injection of a single sperm into the oocyte cytoplasm. PZD involves slicing the outer zona pellucida to create an opening. SUZI deposits sperm directly into the perivitelline space, while enucleation physically removes maternal chromatin from the oocyte.

**130.** (A) II, III and IV
Telomeres are protective capping structures at the ends of chromosomes. In cloned animals, shortened telomeres derived from donor somatic nuclei can lead to premature senescence and aging phenotypes. However, telomerase activity during early embryonic development can actively restore telomere length. Therefore, SCNT does not guarantee perfect telomere length preservation in every clone.

**131.** (A) It allows embryos to auto-condition their environment undisturbed
Single-step media formulations are designed to support embryo development from zygote to blastocyst stage without changing the medium. A primary advantage is that it allows the developing embryos to auto-condition their microenvironment undisturbed. This eliminates the stress of physical handling, pipetting, and fluctuations in temperature and pH during culture.

**132.** (A) Both A and R are true and R is the correct explanation of A.
The generation of induced pluripotent stem cells represents a major ethical advancement in stem cell research. Unlike traditional embryonic stem cells, which require the destruction of early-stage blastocysts to harvest cells from the inner cell mass, iPSCs are reprogrammed directly from adult somatic cells, bypassing ethical concerns regarding embryo destruction.

**133.** (B) An oral progestin to suppress estrus
Melengestrol acetate is an orally active synthetic progestin used to synchronize estrus in heifers. When administered daily in feed, MGA mimics the action of progesterone by exerting strong negative feedback on the hypothalamus, thereby suppressing follicular maturation, behavioral estrus, and ovulation. Upon withdrawal, follicular development resumes, leading to synchronized estrus.

**134.** (C) Polyploidy and embryonic lethality
Polyspermy occurs when more than one sperm cell successfully fertilizes a single oocyte. This abnormal event results in polyploidy, where the zygote contains extra sets of chromosomes. Polyploidy disrupts mitotic spindle formation and early cleavage divisions, leading to severe chromosomal imbalance and inevitable embryonic lethality in mammalian species.

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**135.** (D) The two halves of a bisected embryo are genetically identical
Embryo bisection is a micromanipulation technique where a single embryo, typically a morula or blastocyst, is mechanically split into two equal halves. Because both halves originate from a single fertilized zygote, the resulting demi-embryos are genetically identical, enabling the production of true monozygotic twins upon transfer to recipient animals.

**136.** (A) Osmotic shock and cellular toxicity
Cryoprotectants are added in high concentrations to protect embryos during freezing. Upon thawing, these compounds must be systematically removed through step-wise dilution. Failing to remove them leads to severe osmotic shock, as water rapidly rushes into the cells, causing

**78.** (A) Embryonic stem cells (ESCs) - Trophoblast of the blastocyst
Embryonic stem cells are pluripotent cells capable of differentiating into all embryonic tissues. These cells are isolated specifically from the inner cell mass of the pre-implantation blastocyst, not the trophoblast. The trophoblast is a differentiated outer cell layer that gives rise strictly to extraembryonic placental tissues, making the pairing incorrect.

**79.** (B) Adult stem cells are restricted to certain lineages
Multipotent adult stem cells and pluripotent embryonic stem cells differ significantly in their differentiation potential. Embryonic stem cells can differentiate into any cell type from all three embryonic germ layers. In contrast, adult stem cells are lineage-restricted, meaning they can only generate a limited subset of specialized cell types within their tissue of origin.

**80.** (A) Both A and R are true and R is the correct explanation of A.
Non-surgical embryo recovery in cattle commonly utilizes a flexible, multi-lumen Foley catheter. During flushing, the catheter is guided into the uterine horn, and its integrated balloon is inflated with sterile fluid or air. This inflation seals the lumen of the uterine horn, preventing the flushing medium from leaking out, which ensures high embryo recovery rates.

**81.** (A) Sex chromosome of the oocyte
When selecting oocytes for in vitro maturation, morphologists evaluate structural features associated with developmental competence. Key criteria include a homogeneous ooplasm, the presence of an intact zona pellucida, and multiple compact layers of cumulus cells. Conversely, the sex chromosome carried by the oocyte is entirely irrelevant to its physical maturation capacity in vitro.

**82.** (A) I, II and IV
Embryo splitting is a mechanical micro-procedure that uses a micromanipulator to divide a single embryo into two halves, effectively doubling the embryo count. This results in genetically identical monozygotic twins, not dizygotic twins. While the technique yields identical siblings, the individual pregnancy rates for each halF embryo are typically lower than those of whole embryos.

**83.** (A) Cloprostenol
Cloprostenol is a highly potent synthetic analogue of prostaglandin $F_2$-alpha widely used in veterinary medicine. It acts selectively on the receptors of the corpus luteum to induce rapid luteolysis in cyclic livestock. This regression of luteal tissue reduces progesterone levels, triggering synchronized follicular growth, behavioral estrus, and ovulation within a predictable timeframe.

**84.** (D) Blastomeres flatten to maximize contacts
Compaction occurs at the late morula stage and is the first major morphogenetic transition in mammalian development. Individual spherical blastomeres undergo cellular polarization and flatten against one another, maximizing intercellular contacts. This process is mediated by E-cadherin and results in the formation of tight junctions, creating a sealed outer cell layer.

**85.** (A) P-1, Q-4, R-2, S-3
Different genome editing technologies employ distinct DNA recognition mechanisms. Zinc finger nucleases recognize target sequences via protein domains that bind three to four base pairs. TALENs utilize modular protein domains that bind single nucleotides. CRISPR-Cas9 is guided to target sequences by a single-guide RNA. Homologous recombination relies on sequence homology to introduce genetic changes.

**86.** (C) Producing therapeutic protein in goat milk
Gene pharming refers to the production of medically valuable human therapeutic proteins or industrial enzymes in transgenic livestock. A classic example is expressing a human drug gene under the control of a milk-specific promoter in goats. This directs the synthesis and secretion of the recombinant therapeutic protein directly into the milk for easy harvesting.

**87.** (D) Incomplete donor nucleus chromatin remodeling
Epigenetic reprogramming failures in somatic cell nuclear transfer cloned embryos arise from incomplete chromatin remodeling of the donor somatic nucleus. Somatic cell DNA possesses highly stable methylation and histone modification patterns. The recipient oocyte cytoplasm must erase these tissue-specific marks to restore pluripotency; incomplete erasure results in aberrant developmental gene expression.

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with capacitated spermatozoa to achieve fertilization.

**147.** (A) All chemical components are known
A chemically defined culture medium is a formulation in which all individual chemical constituents and their exact concentrations are precisely known. These media exclude undefined biological fluids, such as serum or follicular fluid, replacing them with recombinant proteins, purified amino acids, and synthetic energy substrates. This high level of standardization improves experimental reproducibility.

**148.** (D) Follicle-stimulating hormone
Follicle-stimulating hormone is the primary gonadotropin used to induce superovulation in cattle. Administering multiple doses of FSH over several days rescues subordinate follicles from undergoing atresia, promoting the growth of multiple dominant follicles. This multi-follicular development culminates in the ovulation of numerous high-quality oocytes ready for subsequent fertilization and embryo recovery.

**149.** (A) P-3, Q-4, R-1, S-2
Mammalian reproductive structures perform highly specialized physiological tasks. The epididymis supports sperm maturation and storage. The corpus luteum produces progesterone to maintain pregnancy. The outer zona pellucida serves to prevent polyspermy and protect the developing early embryo, while the inner cell mass of the blastocyst gives rise to the embryo proper.

**150.** (A) Both A and R are true and R is the correct explanation of A.
Mesenchymal stem cells are widely utilized in veterinary regenerative medicine to treat tendon injuries in horses. These multipotent cells secrete anti-inflammatory cytokines that reduce local tissue inflammation. Furthermore, they can differentiate into functional tenocytes, promoting structural tendon tissue repair and reducing the incidence of scar tissue formation post-injury.

**151.** (D) It causes a high incidence of cystic follicles due to its long halF-life
Equine chorionic gonadotropin possesses a prolonged halF-life in circulation due to its high sialic acid content. This extended bioactivity causes continuous stimulation of ovarian follicles beyond the normal preovulatory window. Consequently, many stimulated follicles fail to ovulate and instead persist, leading to a high incidence of cystic follicles and reduced embryo quality.

**152.** (A) IETS guidelines are universally applied for grading livestock embryos
The International Embryo Technology Society established standardized guidelines that are globally accepted for evaluating and grading livestock embryos. These objective criteria ensure uniformity in research and commercial embryo transfer industries worldwide. Grading relies on morphological features, such as blastomere symmetry, extrusion, and fragmentation levels, rather than simple measurements like diameter.

**153.** (B) To synchronize ovulation to allow for fixed-time artificial insemination
The Ovsynch protocol utilizes sequential administrations of gonadotropin-releasing hormone and prostaglandin $F_2$ alpha to control follicular development and luteolysis. This hormonal regimen tightly synchronizes the timing of ovulation in dairy cows. By coordinating ovulation, dairy producers can perform fixed-time artificial insemination without the need for labor-intensive, continuous estrus detection methods.

**154.** (B) II and III
Mammalian X-bearing sperm contain more DNA than Y-bearing sperm, not less, because the X chromosome is physically larger than the Y chromosome. Flow cytometry successfully exploits this measurable difference in nuclear DNA content, which is approximately three to four percent in cattle, to sort viable sperm cells for artificial insemination or in vitro fertilization.

**155.** (A) ICSI allows fertilization using severe oligozoospermic or dead sperm
Intracytoplasmic sperm injection directly bypasses natural physiological barriers to fertilization by physically microinjecting a single spermatozoon directly into the oocyte cytoplasm. This targeted technique is highly valuable because it achieves fertilization even when using semen samples with extremely low sperm counts, poor motility, or completely non-motile and dead sperm that cannot fertilize standard in vitro setups.

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**98.** (C) Removing the oocyte's chromosomes
Enucleation is a critical step in somatic cell nuclear transfer that involves removing the endogenous maternal chromosomes from the recipient oocyte. This process is typically performed at the metaphase II stage, using a microaspirator to extract the spindle-chromosome complex along with the first polar body, preparing the cytoplasm for the donor nucleus.

**99.** (A) Karyotyping
Karyotyping is the standard cytogenetic technique used to analyze the complete chromosome complement of a cell. By arresting dividing blastomeres at the metaphase stage of mitosis, chromosomes can be stained, visualized, and counted under microscopy. This method directly evaluates the ploidy of a biopsied embryo to detect abnormalities like aneuploidy.

**100.** (A) P-3, Q-2, R-4, S-1
Key events of mammalian reproduction occur in distinct anatomical regions. Sperm capacitation takes place throughout the female reproductive tract. Fertilization occurs specifically at the ampulla-isthmic junction of the oviduct. The early cleavage divisions proceed during embryo transit down the oviduct. Finally, the blastocyst reaches the uterus, where hatching and subsequent implantation occur.

**101.** (B) To penetrate the zona pellucida
The acrosome reaction is a specialized exocytotic process that occurs when a capacitated sperm binds to receptors on the egg's surface. It releases hydrolytic enzymes, such as acrosin, from the sperm head. These enzymes locally digest the protective glycoprotein matrix of the zona pellucida, allowing the sperm to penetrate and fertilize the oocyte.

**102.** (A) Both A and R are true and R is the correct explanation of A.
Teratoma formation is a hallmark functional test used to confirm the pluripotency of stem cells. When pluripotent cells are injected into immunodeficient mice, they lack differentiation-restricting cues. Consequently, they divide and differentiate haphazardly, forming benign tumors containing complex tissues representing all three embryonic germ layers: ectoderm, mesoderm, and endoderm.

**103.** (D) Phytase transgene in pigs - Reduced phosphorus pollution
Monogastric animals like pigs cannot digest phytate phosphorus present in plant feeds, leading to high phosphorus excretion and environmental pollution. Integrating a salivary phytase transgene allows pigs to secrete active phytase enzyme in their saliva. This enzyme breaks down phytate in the digestive tract, improving phosphorus absorption and significantly reducing environmental waste.

**104.** (A) I and III
Follicular aspiration is used to collect immature oocytes for in vitro maturation. This procedure can be performed repeatedly on live animals using ultrasound-guided transvaginal aspiration, also known as ovum pick-up. It physically harvests immature cumulus-oocyte complexes directly from antral follicles, which must subsequently undergo in vitro maturation before fertilization can be attempted.

**105.** (B) To replace cell water and prevent ice crystallization
Permeating cryoprotectants, such as glycerol, ethylene glycol, and dimethyl sulfoxide, have small molecular weights that allow them to cross the cell membrane. During slow-freezing protocols, these molecules enter the cytoplasm, replacing intracellular water. This action decreases the freezing point of the cytoplasm, preventing the formation of lethal ice crystals during cooling.

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**106.** (B) Sperm head alignment during detection
Flow cytometric sorting of semen relies on measuring the fluorescence emitted by stained sperm heads as they pass through a laser beam. Because mammalian sperm heads are flat and highly asymmetric, the fluorescent signal is highly directional. Proper hydrodynamic orientation and alignment of the sperm heads during detection are critical for accurate sorting.

**107.** (A) It is caused by viral infections during pregnancy
Large Offspring Syndrome is a developmental disorder observed in cloned ruminant offspring, characterized by fetal overgrowth, placental abnormalities, and neonatal mortality. This condition is caused by failures in epigenetic reprogramming of the donor genome, which leads to the abnormal expression of imprinted

**165.** (A) I, IV, II, III
Artificial insemination was developed and commercialized first in the mid-twentieth century to improve livestock genetics. In vitro fertilization succeeded it as techniques for culturing gametes matured. Somatic cell nuclear transfer emerged next, marked by the cloning of Dolly the sheep in the late nineties. CRISPR-Cas9 genome editing represents the most modern tool developed recently.

**166.** (C) Rhythmic expansion and contraction of the blastocyst
The mechanical hatching of a blastocyst from its protective glycoprotein shell is driven by dynamic structural changes. Fluid accumulation within the blastocoele cavity increases internal pressure. Coupled with rhythmic pulses of expansion and contraction of the embryo, this pressure stretches and thins the zona pellucida. Enzymatic digestion of the shell eventually permits the embryo to escape.

**167.** (D) Zygote - Single cell formed by syngamy
A zygote is the initial single-cell stage of development created when the male and female haploid pronuclei fuse during fertilization. In contrast, the morula is a solid sphere of cells, while the blastocyst stage is defined by the formation of a fluid-filled cavity. Correct nomenclature is essential to ensure accurate communication and staging in embryology.

**168.** (A) A small molecule like glycerol that crosses the cell membrane
Permeating cryoprotectants are low-molecular-weight chemical compounds, such as glycerol, dimethyl sulfoxide, or ethylene glycol, that readily cross the cell membrane. Once inside the cytoplasm, these agents depress the freezing point, reduce intracellular ice crystal growth, and protect cellular organelles. This action is critical for minimizing mechanical damage during freezing and thawing protocols.

**169.** (A) P-2, Q-1, R-4, S-3
Enucleation is the surgical removal of maternal chromatin from the recipient oocyte. Reconstruction involves inserting the donor somatic cell into the perivitelline space of the enucleated oocyte. Electrofusion uses electrical pulses to merge the somatic cell membrane with the ooplasm. Activation mimics fertilization, using calcium transients to trigger embryonic development and cell division.

**170.** (A) Both A and R are true and R is the correct explanation of A.
Administering a local epidural anesthetic to a donor cow is standard veterinary practice prior to non-surgical embryo flushing. The anesthesia blocks sensory signals in the pelvic region, which relaxes the rectal wall musculature and prevents painful straining. This allows the technician to perform rectal palpation and manipulate the uterine catheter safely without causing discomfort.

**171.** (A) It provides a continuous release of progesterone, mimicking the luteal phase
Controlled Internal Drug Release devices contain progesterone that is slowly absorbed through the vaginal mucosa into the bloodstream. This continuous administration maintains high progesterone levels, effectively mimicking a functional luteal phase. High progesterone suppresses the release of gonadotropins, which prevents premature estrus and ovulation until the device is removed to synchronize the herd.

**172.** (A) I, II and III
Embryonic stem cells can form all embryonic tissues, making them pluripotent. Adult stem cells are restricted to forming a few specialized cell lineages within their tissue of origin, so they are multipotent. Induced pluripotent stem cells share these pluripotency properties, unlike unipotent cells which can only generate one specific, single cell type.

**173.** (D) Extremely high rates of fetal loss and severe placental abnormalities during gestation
Somatic cells possess stable epigenetic marks that must be completely reset by the recipient ooplasm to an embryonic state during cloning. When this epigenetic reprogramming is incomplete, key developmental genes, particularly those regulating placental growth and vascularization, are aberrantly expressed. This failure frequently results in placental malformations, fetal death, and high gestation losses.

**174.** (C) Retroviral vectors only target specific, pre-determined loci in the host genome
Retroviral vectors insert their viral genome randomly into the chromosomes of the host cell rather than targeting specific loci. This random

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oophorus and zona pellucida. This vigorous movement pattern provides the mechanical thrust required for the sperm to navigate the thick cumulus oophorus matrix and successfully penetrate the dense glycoprotein mesh of the zona pellucida during fertilization.

**194.** (C) Interferon-tau
In sheep and cattle, the conceptus secretes interferon-tau as the signaling molecule for maternal recognition of pregnancy. Interferon-tau acts locally on the uterine endometrium to suppress the expression of oxytocin receptors. This blocks the pulsatile release of luteolytic prostaglandin $F_2$ alpha, thereby maintaining the corpus luteum and ensuring continuous progesterone production.

**195.** (B) II and III
Pronuclear microinjection has a low efficiency of transgene integration, typically yielding successful transgenics in less than five percent of cases. Conversely, somatic cell nuclear transfer using pre-transfected donor cells ensures that all resulting cloned offspring stably carry the transgene. Sperm-mediated gene transfer is not considered the most reliable method due to variable and inconsistent results.

**196.** (A) I, II, III, IV
Slow-freezing of embryos begins with the gradual equilibration of the cells in a permeating cryoprotectant like glycerol. The embryos are loaded into straws and cooled, followed by seeding to induce controlled extracellular ice formation. The temperature is decreased at a slow, programmed rate before the straws are plunged into liquid nitrogen.

**197.** (B) It forms the fetal portion of the placenta
The trophectoderm is the outer epithelial cell layer of the blastocyst and represents the first differentiated cell lineage. Its primary biological function is to mediate implantation into the maternal uterine wall and form the fetal portion of the placenta. It does not contribute to the embryo proper, which is derived from the inner cell mass.

**198.** (C) Somatic Cell Nuclear Transfer
Somatic cell nuclear transfer is a specialized cloning technique. The maternal genetic material of an unfertilized metaphase II oocyte is removed during enucleation. A diploid nucleus from a differentiated donor somatic cell is then transferred into this enucleated ooplasm. Upon activation, the oocyte cytoplasm reprograms the somatic nucleus to direct complete embryonic development.

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**199.** (A) P-4, Q-3, R-2, S-1
Slow-freezing depends on gradual dehydration of cells driven by extracellular ice formation. Vitrification uses high cryoprotectant concentrations and rapid cooling to achieve an ice-free, glass-like state. Seeding involves inducing controlled ice formation at specific temperatures to avoid supercooling. Thawing is the physiological transition of frozen biological material back to its liquid state.

**200.** (A) Cleavage medium (Day 1-3) followed by Blastocyst medium (Day 4-8)
In vitro embryo development is optimized using sequential culture media that mimic the changing environments of the oviduct and uterus. Freshly fertilized zygotes are cultured in cleavage medium during the first three days, which is low in glucose. Embryos are then shifted to blastocyst medium from days four to eight to support energy needs.

**201.** (B) To create targeted double-strand breaks for specific genome editing
Zinc finger nucleases are artificial restriction enzymes designed to bind specific DNA sequences. Their primary purpose is to introduce targeted double-strand breaks at precise genomic loci within the host cell. The cellular repair of these breaks allows for highly specific genome editing, including gene disruption or precise gene insertions in livestock breeding programs.

**202.** (A) Both A and R are true and R is the correct explanation of A.
Cloned embryos produced via somatic cell nuclear transfer frequently exhibit incomplete epigenetic reprogramming. Differentiated somatic nuclei possess highly stable, tissue-specific chromatin structures stabilized by extensive DNA methylation and histone modifications. The recipient oocyte cytoplasm often struggles to fully erase these somatic marks and reset the nuclear chromatin to a completely pluripotential state.

**184.** (A) An animal engineered to produce human pharmaceuticals, typically in its milk
A bioreactor animal is a transgenic animal genetically engineered to express complex human therapeutic proteins, usually within its mammary glands. The target proteins are secreted directly into the milk during lactation, which allows for straightforward harvesting and purification. This system provides a cost-effective and highly scalable platform for producing complex pharmaceutical proteins.

**185.** (B) It introduces undefined variables and carries a risk of viral contamination
Fetal bovine serum is commonly added to culture media to provide nutrient support. However, it is an undefined biological product with significant batch-to-batch composition variability. Its use introduces unknown components into the system and carries a risk of transmitting viral pathogens or prions. This complicates the standardization of culture protocols and regulatory approvals.

**186.** (A) Both A and R are true and R is the correct explanation of A.
Under the International Embryo Technology Society guidelines, Grade 1 embryos represent the highest quality category, categorized as excellent. These embryos are selected because they demonstrate symmetrical, uniform blastomeres that match their developmental stage. Furthermore, they contain minimal extracellular debris, showing less than ten percent cellular fragmentation, which correlates with high pregnancy rates.

**187.** (A) To release hydrolytic enzymes that digest the zona pellucida
The acrosome reaction is an essential exocytotic process triggered when the sperm binds to the zona pellucida. This reaction fuses the outer acrosomal membrane with the sperm plasma membrane, releasing stored hydrolytic enzymes like acrosin and hyaluronidase. These enzymes locally digest the extracellular glycoprotein matrix, allowing the sperm to penetrate to the oolemma.

**188.** (A) P-4, Q-2, R-3, S-1
In vitro fertilization often suffers from high rates of polyspermy due to excessive sperm-to-oocyte ratios. Somatic cell nuclear transfer is limited by low efficiency and developmental abnormalities like Large Offspring Syndrome. Pronuclear microinjection results in random integration and variable transgene expression. Flow cytometry is constrained by slow sorting speeds and damage to sperm membranes.

**189.** (A) I, II and IV
CRISPR-Cas9 works by inducing targeted double-strand breaks at specific locations in the genome. It utilizes a designed guide RNA sequence to direct the Cas9 nuclease to its target site, avoiding random viral integration. The cellular repair pathways then enable researchers to achieve precise gene knockouts or site-specific insertions of donor DNA sequences.

**190.** (C) Differentiation into an inner cell mass and a trophectoderm layer surrounding a fluid cavity
The blastocyst stage represents the first lineage differentiation event during early mammalian development. It is structurally characterized by the formation of a fluid-filled cavity called the blastocoele. This cavity is surrounded by an outer monolayer of trophectoderm cells, with a distinct, compact group of cells known as the inner cell mass situated at one pole.

**191.** (B) Prolonged exposure to ultraviolet light is harmless to embryos
During embryo transfer procedures, maintaining embryo viability requires strict control over environmental conditions. Embryos are highly sensitive to physical stress and must be kept at physiological temperatures and stable pH values. Exposure to ultraviolet light is highly damaging, as it induces DNA lesions and oxidative stress, which quickly compromises embryo development and survival.

**192.** (D) To prevent mechanical damage from intracellular ice crystal formation
Dimethyl sulfoxide is a permeating cryoprotectant that readily penetrates the cell membrane. Its main role is to lower the freezing point of the cytoplasm and reduce the formation of intracellular ice crystals during cooling. By replacing intracellular water, it protects sensitive cell membranes and organelles from mechanical damage caused by sharp ice growth.

**193.** (A) Both A and R are true and R is the correct explanation of A.
Capacitated sperm display hyperactivated motility. Hyperactivation provides the mechanical force necessary to penetrate the cumulus

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**232.** (A) The maintenance of gap junctions between the cumulus cells and the oocyte
The successful in vitro maturation of oocytes depends heavily on the maintenance of functional gap junctions between the surrounding cumulus cells and the oocyte. These intercellular channels are essential for transmitting nutrients, metabolic intermediates, and signaling molecules like cyclic AMP, which coordinate the biochemical events of cytoplasmic and nuclear maturation.

**233.** (B) It solely affects the physical size of the offspring with no effect on internal organs
Large Offspring Syndrome is an epigenetic condition caused by embryo manipulation. It does not solely affect physical size; affected offspring also display severe internal organ abnormalities, such as cardiomegaly, hepatomegaly, and respiratory distress. These developmental defects stem from the misregulation of imprinted genes like $IGF_2$, which compromises organogenesis and reduces postnatal survival.

**234.** (A) Both A and R are true and R is the correct explanation of A.
CRISPR-Cas9 has greatly improved the efficiency of producing gene-edited livestock compared to traditional homologous recombination. CRISPR-Cas9 utilizes a customizable guide RNA to introduce site-specific double-strand breaks in the target DNA. These breaks stimulate the cell's endogenous DNA repair mechanisms, increasing the frequency of gene modification by several orders of magnitude.

**235.** (A) To maximize the number of viable embryos produced in a single estrous cycle
The primary purpose of superovulation in donor animals is to maximize the yield of viable embryos during a single estrous cycle. Administering exogenous gonadotropins rescues follicles that would otherwise undergo atresia, allowing multiple follicles to mature and ovulate. This enables the non-surgical recovery of many embryos for transfer into recipient surrogates.

**236.** (A) I and II
Immunological assays for embryo sexing are based on detecting male-specific H-Y cell surface antigens. These assays require binding specific anti-H-Y antibodies to the embryo, which are then detected using a secondary fluorescent antibody. However, these immunological methods are generally considered less accurate and reliable compared to highly sensitive PCR-based sexing techniques.

**237.** (D) Sperm-mediated gene transfer
Sperm-mediated gene transfer is a transgenesis technique that exploits the natural ability of mature sperm cells to bind and internalize exogenous DNA. During fertilization, these transfected sperm act as vectors to deliver the foreign genetic material directly into the ooplasm of the oocyte, facilitating its integration into the newly forming embryonic genome.

**238.** (C) It must undergo capacitation to destabilize its plasma membrane
Before a sperm cell can undergo the acrosome reaction, it must undergo capacitation within the female reproductive tract. This biochemical process removes seminal proteins, alters membrane cholesterol, and increases calcium permeability. These changes destabilize the sperm plasma membrane, priming it to respond to zona pellucida glycoproteins and release its acrosomal enzymes.

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**239.** (A) II, I, IV, III
Early embryo development begins with syngamy, which is the fusion of parental pronuclei to form a diploid zygote. Cleavage divisions proceed until compaction occurs at the morula stage, where blastomeres flatten. This is followed by blastulation, forming a fluid-filled cavity, and culminates in hatching from the protective zona pellucida.

**240.** (A) P-2, Q-4, R-1, S-3
Somatic cell nuclear transfer manipulates the recipient ooplasm and donor somatic nucleus. Intracytoplasmic sperm injection involves introducing a single spermatozoon directly into the ooplasm. Pronuclear microinjection targets the male and female pronuclei of a zygote. Partial Zona Dissection involves mechanically slicing the glycoprotein-based zona pellucida of an unfertilized oocyte.

**241.** (A) The dominant follicle secretes inhibin, depressing FSH levels and starving smaller follicles
The dominant follicle achieves selection and suppresses competitors by secreting high levels of estrogen and inhibin. Inhibin directly acts on the anterior pituitary to suppress the synthesis and release of follicle-stimulating hormone.

**203.** (A) Extrusion of the first polar body
An unfertilized metaphase II oocyte has completed the first meiotic division, which is morphologically marked by the extrusion of the first polar body into the perivitelline space. At this stage, the genetic material remains arrested in the second meiotic metaphase, awaiting fertilization. Pronuclei only form after sperm entry triggers the completion of meiosis.

**204.** (B) III only
Stage-specific embryonic antigens are key cell-surface glycolipids, and Nanog is a crucial nuclear transcription factor involved in pluripotency. Oct-4 is expressed specifically in the undifferentiated inner cell mass of the blastocyst but is down-regulated in the trophectoderm. Stem cell marker expression decreases as cells commit to specific lineages and undergo differentiation.

**205.** (D) They act as an osmotic buffer to safely draw water out of the cell
Non-permeating cryoprotectants, such as sucrose, cannot cross the cell membrane and remain in the extracellular space. This creates an osmotic gradient that draws water out of the cells, aiding in cellular dehydration prior to freezing. During thawing, sucrose acts as an osmotic buffer to prevent excessive, rapid water influx and subsequent osmotic lysis.

**206.** (C) Micromanipulation and bisection of a single morula
Bisection of a high-quality morula or early blastocyst using a microblade is the most effective method to produce monozygotic twins. This micromanipulation splits the embryo into two equal halves, which are then transferred into recipient surrogates. Because both halves originate from a single fertilized zygote, the resulting calves are genetically identical twins.

**207.** (B) Transfer catheter - Instrument used to deposit embryos into the uterine horn
An embryo transfer catheter is a specialized, flexible instrument designed to safely deposit the selected embryo into the lumen of the uterine horn contralateral to the active corpus luteum. Foley catheters are used for non-surgical embryo recovery rather than surgical removal. Epidural anesthesia is administered to the donor cow, not directly to the embryo.

**208.** (D) The period of sexual receptivity driven by peak estrogen levels
Estrus is a distinct phase of the female reproductive cycle characterized by behavioral and physiological signs of sexual receptivity. This period of standing heat is driven by high concentrations of estrogen secreted by mature Graafian follicles in the ovary. Peak estrogen levels act on the brain to stimulate characteristic behaviors that permit mating.

**209.** (A) II, III, I, IV
The chronological sequence of events starts with the onset of estrus, which is driven by rising estrogen levels. This behavioral heat triggers the preovulatory luteinizing hormone surge from the pituitary gland. The LH surge induces ovulation, releasing the mature oocyte. Insemination provides capacitated sperm that fertilize the egg, completing fertilization.

**210.** (A) P-4, Q-2, R-3, S-1
Transgenic technology can enhance production traits, such as engineering sheep for increased wool growth. Gene pharming is exemplified by goats producing human antithrombin in their milk. Disease resistance is achieved by engineering cattle resistant to mastitis. Environmental mitigation includes pigs expressing phytase to reduce the levels of polluting phosphorus in their manure.

**211.** (A) Both A and R are true and R is the correct explanation of A.
During the luteal phase of the estrous cycle, wave-like follicular development continues, and a dominant follicle will successfully emerge. However, because progesterone levels remain high, the hypothalamus and pituitary are inhibited, suppressing the preovulatory LH surge. Lacking this essential luteinizing hormone support, the dominant follicle cannot ovulate and eventually undergoes atresia.

**212.** (D) Adult stem cells are multipotent and naturally occurring, while iPSCs are reprogrammed to be pluripotent
Adult stem cells reside naturally within specific tissue niches in the body and are multipotent, meaning they can only differentiate into a limited number of closely related cell types. In contrast, induced pluripotent stem cells are artificial, reprogrammed from differentiated somatic

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cells using transcription factors to achieve a pluripotent state similar to embryonic stem cells.

**213.** (B) I, III and IV
Sex-sorting achieves high purity, typically ninety percent or greater. The process uses a DNA-binding fluorochrome to differentiate X and Y sperm. However, the physical stress of staining and flow cytometry sorting decreases sperm viability and lifespan, resulting in lower conception rates compared to using conventional, unsorted semen in commercial breeding.

**214.** (C) It is an acellular glycoprotein matrix essential for species-specific sperm binding
The zona pellucida is a specialized, acellular extracellular matrix composed of glycoproteins that completely surrounds the oocyte. It is synthesized during follicular growth and plays a critical role in species-specific sperm recognition, binding, and induction of the acrosome reaction. After fertilization, it is biochemically altered to prevent polyspermy and remains intact during early cleavage.

**215.** (D) Integration occurs randomly, leading to variable expression and potential insertional mutagenesis
Pronuclear microinjection introduces foreign DNA directly into the pronucleus of a zygote. A major disadvantage is that the transgene integrates randomly into the host genome. This random integration can disrupt native genes, leading to insertional mutagenesis, or place the transgene in transcriptionally inactive regions, resulting in highly variable expression levels in offspring.

**216.** (A) Secretion of estrogen by the conceptus
In pigs, maternal recognition of pregnancy is initiated by the secretion of estrogen, specifically estradiol, by the developing blastocysts. This hormonal signal reroutes the secretion of luteolytic prostaglandin $F_2$ alpha from an endocrine pathway toward the uterine lumen, where it is deactivated. This prevents luteolysis, preserving the corpus luteum for progesterone synthesis.

**217.** (B) Embryonic stem cells are currently the routine standard of care in all commercial livestock operations
While mesenchymal stem cells are widely used in veterinary medicine to treat osteoarthritis, embryonic stem cells are not the routine standard of care in commercial livestock operations. Deriving and maintaining true embryonic stem cells in ungulates is highly challenging. Consequently, their use is currently restricted to specialized laboratory research rather than routine field operations.

**218.** (D) A is false but R is true.
Early cleavage stage embryos are generally easier to evaluate individually because their blastomeres are distinct and separate. In contrast, evaluating a compact morula is more challenging. During compaction, the blastomeres coalesce into a tight mass, which can mask cellular fragmentation and degeneration, making accurate morphologic grading of the embryo more difficult.

**219.** (A) Deflection of charged droplets containing individual sperm based on their relative fluorescence
Semen sorting via flow cytometry relies on staining sperm with a DNA-specific fluorescent dye. Because X-bearing sperm contain more DNA, they emit stronger fluorescence when passing through a laser. The instrument generates charged droplets containing individual sperm and uses electrostatic deflection plates to sort them into different collection tubes based on this fluorescence intensity.

**220.** (A) P-2, Q-4, R-1, S-3
A gene knockout involves the disruption or inactivation of an existing gene. A gene knock-in is the targeted insertion of a specific new genetic sequence. CRISPR-Cas9 is characterized by its use of a guide RNA to achieve site-specific DNA cleavage. Retroviral integration is characterized by random insertion biased toward transcriptionally active areas.

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**221.** (A) I and II
During early cleavage, the total embryonic mass decreases slightly as cells divide within the zona pellucida without growth. Early development is controlled by maternal mRNA stored in the oocyte. The transition to embryonic genomic control does not occur immediately at fertilization, but rather at species-specific stages, such as the eight-cell stage in cattle.

**222.** (C) Binding of the sperm to the ZP3 glycoprotein
In vivo, the acrosome reaction is directly triggered when the capacitated sperm cell binds to the ZP3

glycoprotein receptor on the zona pellucida. This binding event initiates intracellular signaling cascades within the sperm head, leading to an influx of calcium ions. This calcium surge drives the exocytosis of acrosomal enzymes to digest the zona matrix.

**223.** (B) It directs the expression of the transgene specifically to the mammary gland during lactation
The beta-lactoglobulin promoter is a tissue-specific regulatory element. In gene pharming, fusing this promoter to a therapeutic transgene ensures that the target protein is expressed specifically in the mammary epithelial cells during lactation. This targeted expression restricts protein secretion to the milk, protecting the animal's systemic health from any bioactivity of the transgene.

**224.** (D) IVF - Co-incubation of mature fertilizable oocytes and fully capacitated sperm
In vitro fertilization is defined as the co-incubation of mature oocytes with capacitated sperm in a controlled droplet to achieve fertilization. In contrast, in vitro maturation involves maturing immature germinal vesicle oocytes to metaphase II. In vitro culture refers to growing newly fertilized zygotes to the blastocyst stage, and ICSI involves injecting a single sperm.

**225.** (A) Aberrant placental development stemming from improper genomic imprinting
Cloned embryos produced via somatic cell nuclear transfer often suffer from improper genomic imprinting, which leads to the misregulation of key development genes. This epigenetic defect causes abnormal placental development, characterized by reduced vascularization and cotyledon development. The compromised placenta fails to sustain the fetus, triggering pregnancy loss and rejection by the surrogate mother.

**226.** (A) Both A and R are true and R is the correct explanation of A.
Vitrification media are formulated with significantly higher concentrations of cryoprotectants compared to slow-freezing media. These high concentrations are necessary to increase the viscosity of the solution. When cooled rapidly, this high viscosity prevents water molecules from organizing into crystalline structures, ensuring the sample solidifies into an amorphous, glass-like state.

**227.** (A) Mechanical breaching of the zona pellucida to give sperm direct access to the oolemma
Partial Zona Dissection is a micromanipulation technique used to assist fertilization in cases of poor semen quality. It involves mechanically breaching the outer zona pellucida by creating a small slit using a specialized glass micro-needle. This physical opening allows sperm to bypass the dense glycoprotein matrix and directly access the oolemma to achieve fertilization.

**228.** (B) III only
The luteal phase is characterized by a functional corpus luteum, which is regressed by uterine prostaglandin $F_2$ alpha. The follicular phase is dominated by estrogen secreted by growing follicles, not progesterone, which is low during this phase. Estrus behavior coincides with peak estrogen levels, which trigger the preovulatory luteinizing hormone surge.

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**229.** (A) II, I, IV, III
Somatic cell cloning begins with synchronizing the donor cells, typically to the $G_0$ or $G_1$ stage of the cell cycle. The recipient oocyte is then enucleated to remove its maternal genetic material. The donor somatic cell is introduced and fused with the enucleated oocyte via electrofusion, followed by artificial activation of the cloned embryo.

**230.** (A) P-2, Q-1, R-4, S-3
Oct-4 is a crucial transcription factor required for maintaining pluripotency. CD44 and CD90 are surface antigens used to identify mesenchymal stem cells. Alkaline phosphatase is an enzyme expressed in undifferentiated embryonic stem cells. MyoD is a key marker representing commitment of cells to the skeletal muscle differentiation lineage.

**231.** (D) The two halves are derived from the same fertilized zygote and share identical DNA
Embryo bisection involves dividing a single morula or blastocyst into two halves using a microblade. Because both halves are derived from the same original fertilized zygote, they share an identical genomic sequence. When transferred into suitable surrogate mothers, each half retains the developmental potential to form a complete, genetically identical individual.

integration can disrupt functional genes or result in poor transgene expression. Conversely, somatic cell nuclear transfer using pre-screened, transfected cells allows researchers to verify stable and precise gene integration prior to creating cloned livestock.

**175.** (B) It requires moving embryos, exposing them to environmental stress
Sequential media systems are designed to match the changing nutritional requirements of the developing embryo as it moves along the reproductive tract. However, this approach requires physically transferring the embryos between different culture droplets. This handling exposes the sensitive embryos to potential fluctuations in temperature, pH, and osmolarity, which can induce cellular stress.

**176.** (D) Molecular screening via PCR and sequencing
Confirming that a specific gene has been successfully knocked out or modified in a livestock embryo requires direct analysis of its DNA sequence. Visual evaluation of morphology or viability under a microscope cannot detect precise genomic alterations. Molecular techniques like polymerase chain reaction followed by DNA sequencing are essential to verify targeted mutations.

**177.** (A) P-3, Q-1, R-2, S-4
Gonadotropin-releasing hormone acts on the pituitary gland to trigger ovulation or luteinize active follicles. Prostaglandin $F_2$ alpha acts on the ovary to induce regression of the corpus luteum. Exogenous progestins, like CIDR inserts, function to suppress estrus behavior. Equine chorionic gonadotropin acts similarly to FSH to stimulate the growth of ovarian follicles.

**178.** (A) Both A and R are true and R is the correct explanation of A.
Sperm capacitation is a reversible physiological process. Ejaculated sperm undergo biochemical modifications in the female reproductive tract that remove coating proteins and alter the plasma membrane to permit fertilization. If these capacitated sperm are returned to seminal plasma, decapacitation factors bind back to the sperm membrane, reversing the capacitation process until they are re-exposed.

**179.** (A) Cleavage involves cell divisions without any increase in total cytoplasmic mass
Early embryonic cleavage is characterized by rapid mitotic divisions that occur within the rigid boundaries of the zona pellucida. Unlike typical somatic cell mitosis, there is no cell growth phase between successive divisions. Consequently, the total cytoplasmic volume of the embryo remains constant, and each division halves the size of the resulting daughter blastomeres.

**180.** (A) I only
Cumulus cells do not block sperm penetration; instead, they assist fertilization by releasing chemoattractants and factors that facilitate capacitation. These cells also provide physical support during sperm binding and penetration. For these reasons, maintaining intact cumulus investments leads to significantly higher fertilization rates in standard in vitro systems compared to using completely denuded oocytes.

**181.** (B) Cortical granules
Cortical granules are specialized membrane-bound secretory organelles located beneath the oocyte plasma membrane. Upon sperm penetration, a wave of intracellular calcium triggers the exocytosis of these granules. They release hydrolytic enzymes into the perivitelline space, which modify the zona pellucida glycoproteins to permanently block the binding and entry of additional sperm.

**182.** (C) Embryonic stem cells - Inner cell mass
Embryonic stem cells are pluripotent cells that are derived directly from the inner cell mass of pre-implantation blastocysts. These cells can differentiate into all three germ layers. In contrast, mesenchymal stem cells originate from mesodermal tissues, spermatogonial stem cells are found in the testes, and induced pluripotent stem cells are reprogrammed from somatic cells.

**183.** (A) III, IV, II, I
Superovulation in cattle begins with multiple, twice-daily injections of follicle-stimulating hormone to rescue and grow a large cohort of follicles. Prostaglandin $F_2$ alpha is then administered to induce regression of the corpus luteum and initiate estrus. The cow is inseminated during standing heat, and the resulting embryos are flushed non-surgically seven days later.

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This drop in circulating FSH deprives smaller, subordinate follicles of essential survival signals, causing them to undergo atresia.

**242.** (A) Both A and R are true and R is the correct explanation of A.
Generating induced pluripotent stem cells traditionally involves using retroviral or lentiviral vectors to deliver transcription factors. Because these viral vectors integrate randomly into the host cell genome, they carry a risk of disrupting functional genes or activating oncogenes. This potential for insertional mutagenesis represents a major limitation for their clinical application.

**243.** (A) To prevent supercooling by inducing controlled extracellular ice formation
Seeding is a critical step in slow-rate cryopreservation that involves touching the freezing straw with a chilled instrument. This induces controlled ice crystal formation in the extracellular medium. By initiating ice growth, seeding prevents dangerous supercooling and ensures gradual cellular dehydration, which protects the embryo from intracellular ice crystal damage.

**244.** (B) III only
Embryo splitting is most successful at the morula or blastocyst stages and requires a precise micromanipulator setup. The microblade must be aligned to divide the inner cell mass evenly. However, splitting does not guarantee two live births. The manipulation can reduce embryo viability, resulting in lower pregnancy rates per halF-embryo transferred.

**245.** (B) An animal containing populations of cells derived from different zygotes
A chimeric animal is an individual that contains two or more distinct populations of genetically different cells. This condition is created experimentally by merging blastomeres from different early-stage embryos or by injecting pluripotent stem cells into a host blastocyst. The resulting organism displays tissues derived from both contributing zygotic lineages.

**246.** (C) It acts similarly to LH, inducing ovulation of mature follicles
Human chorionic gonadotropin possesses strong luteinizing hormone-like activity in livestock due to its ability to bind the LH receptor. Administering hCG mimics the preovulatory luteinizing hormone surge, which induces the final maturation and ovulation of mature follicles. It is commonly used in estrus synchronization and superovulation protocols to control the timing of ovulation.

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**247.** (D) Acrosin - Enzyme facilitating penetration of the zona pellucida
Acrosin is a key serine protease stored as a zymogen within the sperm acrosome. Upon activation during the acrosome reaction, it is released to locally digest the glycoprotein matrix of the zona pellucida. This enzymatic digestion is essential for allowing the sperm cell to penetrate the outer shell and reach the oocyte plasma membrane.

**248.** (A) Lack of adequate cytoplasmic maturation and stored maternal RNA
Oocytes harvested from small, subordinate follicles display reduced developmental competence because they have not completed cytoplasmic maturation. During follicular growth, the oocyte must accumulate essential proteins, organelles, and maternal RNA transcripts. Lacking these stored maternal components, the oocyte cannot support fertilization, early cleavage, or the transition to embryonic genomic control.

**249.** (A) Both A and R are true and R is the correct explanation of A.
Trehalose is a non-permeating disaccharide used as an extracellular cryoprotectant. Because it cannot cross the cell membrane, it remains in the extracellular medium and creates an osmotic gradient. This gradient draws water out of the cytoplasm, aiding in cellular dehydration. Dehydration is essential for minimizing the formation of damaging intracellular ice crystals.

**250.** (A) P-3, Q-4, R-1, S-2
Heparin is added to IVF media to act as a capacitation agent for bovine sperm. TALP or BO medium provides the necessary nutrient environment for gamete co-incubation. A mineral oil overlay is used to prevent medium evaporation and temperature fluctuations. Carbon dioxide incubators maintain physiological pH by buffering the bicarbonate-based culture media.

**251.** (A) The somatic epigenetic marks are erased and replaced with embryonic marks
Somatic cell nuclear reprogramming is an epigenetic process. The differentiated somatic

nucleus is exposed to the ooplasm, which erases its tissue-specific epigenetic marks, such as DNA methylation and histone modifications. These are replaced with embryonic marks, reverting the somatic gene expression pattern back to a pluripotent state capable of directing development.

**252.** (B) III only
Gene knockouts involve disrupting a specific gene sequence using homologous recombination or modern nucleases to study gene function. While they are highly valuable for research, they do not guarantee enhanced growth in livestock. Some knockouts can cause embryonic lethality, developmental defects, or reduce growth rates depending on the target gene's function.

**253.** (D) A cascade of events modifying the zona pellucida and oolemma to prevent multiple sperm entry
Polyspermy block is a physiological defense mechanism that prevents more than one sperm from fertilizing the oocyte. The entry of the first sperm triggers an intracellular calcium wave, which drives cortical granule exocytosis. These granules release enzymes that biochemically modify the zona pellucida and oolemma, permanently blocking subsequent sperm binding and entry.

**254.** (A) III, I, II, IV
Pronuclear microinjection begins by superovulating and mating donor females to produce single-cell zygotes. These fertilized zygotes are then collected, and the foreign DNA construct is microinjected into the male pronucleus. After microinjection, the zygotes are transferred into synchronized pseudopregnant recipient females to complete gestation and development.

**255.** (B) It can only form one specific cell type, though it retains the ability to selF-renew
A unipotent stem cell is a specialized adult stem cell that can only differentiate into one specific cell type. Despite this narrow differentiation potential, it maintains the defining stem cell property of selF-renewal through mitotic divisions. These cells play a crucial role in the continuous regeneration and repair of highly specialized adult tissues.

**256.** (D) It amplifies highly conserved, sex-specific DNA sequences such as the SRY gene
Polymerase chain reaction-based embryo sexing is a highly accurate molecular technique. It involves taking a small biopsy of cells, typically from the trophectoderm, and extracting their DNA. PCR is then used to amplify highly conserved, male-specific gene sequences located on the Y chromosome, such as the SRY gene, to determine the embryo's sex.

**257.** (A) Both A and R are true and R is the correct explanation of A.
The timing of oocyte activation is a critical determinant of cloning success. If the reconstructed embryo is activated prematurely before chromatin remodeling can occur, the spindle apparatus may fail to coordinate the donor chromosomes. This can result in chromosomal extrusion, aneuploidy, and subsequent developmental arrest of the cloned embryo.

**258.** (C) They are located in specific niches within tissues and contribute to homeostasis and repair
Adult stem cells, also known as somatic stem cells, are undifferentiated cells found in post-natal tissues. They reside within specialized tissue microenvironments called niches. Their primary function is to maintain tissue homeostasis and mediate regeneration and repair by dividing to replace damaged or dying cells within their tissue of origin.

**259.** (A) P-3, Q-1, R-4, S-2
Follicle-stimulating hormone targets the ovaries to stimulate the growth of follicles. Prostaglandin $F_2$ alpha acts on the corpus luteum to induce luteolysis. Oxytocin targets the smooth muscle of the uterus and mammary gland. Gonadotropin-releasing hormone acts on gonadotrophs in the anterior pituitary to regulate LH and FSH release.

**260.** (A) I, II and IV
Intracytoplasmic sperm injection directly introduces the sperm into the cytoplasm, bypassing the acrosome reaction. Partial Zona Dissection creates a slit in the zona pellucida, unlike subzonal insertion which deposits sperm into the perivitelline space. These micro-assisted technologies are frequently used to overcome severe semen abnormalities and improve fertilization rates.

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**261.** (B) Sequential media require changing formulations, while single-step media use one continuous formulation
Sequential culture media systems utilize different formulations that are changed at specific stages to match the embryo's evolving metabolism. Conversely, single-step media systems use a single, continuous formulation throughout the entire culture period. This continuous system relies on the embryo's ability to selectively upscale nutrients without the stress of manual transfer.

**262.** (C) CRISPR-Cas9
CRISPR-Cas9 is the most efficient method for introducing targeted double-strand breaks at precise genomic locations. Unlike pronuclear microinjection or retroviral integration, which insert DNA randomly, CRISPR-Cas9 utilizes a guide RNA to direct the Cas9 nuclease to a specific target sequence. This allows for highly precise gene disruption or editing in host cells.

**263.** (D) True, validated ES cells have been remarkably easy to derive and maintain in ungulates compared to mice
While embryonic stem cells hold theoretical potential for transgenesis and disease modeling, deriving and maintaining validated ES cell lines in ungulates is extremely difficult. Unlike in mice, livestock pre-implantation blastocysts do not readily yield stable, pluripotent ES cell lines in standard culture. This challenge has limited their commercial application in animal biotechnology.

**264.** (A) Both A and R are true and R is the correct explanation of A.
Progestin-based synchronization protocols, such as long-term CIDR treatments, achieve tight estrus synchronization but can lead to reduced conception rates. Prolonged exposure to exogenous progesterone suppresses the preovulatory LH surge, which causes the dominant follicle to persist. This persistent follicle ages and contains an oocyte with reduced developmental competence upon ovulation.

**265.** (B) iPSCs circumvent ethical concerns of embryo destruction and allow for autologous therapies
Induced pluripotent stem cells offer major clinical advantages over embryonic stem cells by bypassing the ethical concerns associated with destroying pre-implantation embryos. Furthermore, because iPSCs can be derived from the patient's own somatic cells, they enable the creation of patient-specific, autologous cellular therapies that do not trigger host immune rejection upon transplantation.

NODIA

**266.** (B) I, II, III, IV
Embryo transfer in cattle begins with the administration of local epidural anesthesia to relax the rectum and genital tract. Rectal palpation is performed next to locate the active corpus luteum. The selected embryo is then loaded into the transfer catheter, which is carefully guided to deposit the embryo into the ipsilateral uterine horn.

**267.** (A) P-2, Q-3, R-4, S-1
Primordial follicles feature an oocyte surrounded by a single layer of squamous cells. Primary follicles feature an oocyte surrounded by cuboidal granulosa cells. Antral follicles are characterized by the initial formation of a fluid-filled cavity called the antrum. Graafian follicles represent the fully developed preovulatory stage ready to undergo ovulation.

**268.** (B) V only
Vitrification requires ultra-rapid cooling and high concentrations of cryoprotectants to prevent ice crystal formation, making it the preferred method for cryopreserving oocytes. However, vitrification does not require a programmable freezer. Instead, the samples are plunged directly into liquid nitrogen to achieve the fast cooling rates necessary for glass-like solidification.

**269.** (D) RNA-DNA base pairing guided by an sgRNA
The targeting specificity of the CRISPR-Cas9 system is driven by direct Watson-Crick base pairing between a designed single guide RNA and the target genomic DNA. The guide RNA sequence aligns with the complementary DNA sequence, bringing the Cas9 nuclease into close proximity to induce a precise, site-specific double-strand break.

**270.** (C) The biochemical changes sperm undergo in the female tract rendering them competent to fertilize
Capacitation is a series of biochemical and physiological modifications that ejaculated sperm must undergo within the female reproductive tract to gain fertilizing ability. This process destabilizes the sperm plasma membrane, alters

cholesterol content, and increases membrane permeability to calcium. These changes enable the sperm to undergo the acrosome reaction and exhibit hyperactivated motility.

**271.** (B) Grade 1 - Perfect embryo, minimal fragmentation
In embryo grading, Grade 1 represents the highest quality category, characterized by symmetrical blastomeres and little to no cellular fragmentation. Grade 2 embryos display moderate irregularities, Grade 3 embryos exhibit prominent fragmentation, and Grade 4 embryos are dead or severely degenerated. Consequently, Grade 1 embryos are the most ideal candidates for cryopreservation.

**272.** (A) Both A and R are true and R is the correct explanation of A.
Large Offspring Syndrome is a developmental abnormality frequently observed in somatic cell clones. The in vitro manipulation of gametes and somatic cell nuclear transfer can induce epigenetic instability, leading to the loss of genomic imprinting. This results in the misregulation of key development genes, such as $IGF_2$, which drives fetal overgrowth and skeletal malformations.

**273.** (A) Prostaglandin $F_2$ alpha
Prostaglandin $F_2$ alpha is a luteolytic hormone synthesized by the uterine endometrium during the late luteal phase. Exogenous administration of Prostaglandin $F_2$ alpha binds to receptors on luteal cells, inducing luteolysis and regression of the corpus luteum. This regression causes a rapid decline in progesterone, allowing the animal to return to estrus.

**274.** (B) I and III
Pronuclear microinjection is a highly demanding and labor-intensive micro-manipulation technique. A key limitation is that it yields a very low success rate of producing stable, functional transgenic animals. This is because the foreign DNA integrates randomly into the host genome, preventing researchers from controlling the exact site of transgene integration.

**275.** (A) To supply a microenvironment providing signals that maintain selF-renewal and multipotency
The stem cell niche is a specialized, local microenvironment that surrounds stem cells within tissues. Its fundamental role is to provide physical support and transmit extracellular signals, such as growth factors and cell-cell contacts, that prevent differentiation. This signaling network maintains the stem cells in an undifferentiated, selF-renewing, and multipotent state.

**276.** (A) II, III, IV, I
Fertilization begins when the capacitated sperm binds to the zona pellucida of the oocyte. This binding induces the acrosome reaction, releasing enzymes that digest the zona. Sperm-oocyte membrane fusion triggers the cortical reaction to block polyspermy. Finally, the male and female haploid pronuclei fuse during syngamy, forming a diploid zygote.

**277.** (A) P-2, Q-1, R-4, S-3
Pronuclear microinjection is limited by random integration and position effects. Traditional homologous recombination suffers from extremely low targeting efficiency in somatic cells. Retroviral vectors have limited packaging capacity and are subject to transgene silencing. CRISPR-Cas9 is a highly efficient editing tool but carries a potential risk of inducing ofF-target mutations.

**278.** (C) They utilize a monolayer of cells to support embryo development in vitro
Somatic cell co-culture systems utilize a supporting feeder monolayer, such as bovine oviductal epithelial cells, to support early embryo development in vitro. These feeder cells metabolize embryotoxic components, secrete beneficial growth factors, and optimize the nutrient composition of the culture medium, thereby improving blastocyst development rates in vitro.

**279.** (A) Both A and R are true and R is the correct explanation of A.
Embryo bisection is a viable and commercial micromanipulation technique used to produce genetically identical twin calves. Early embryonic blastomeres, prior to committing to specific cell lineages, possess high developmental plasticity. When an embryo is split, the remaining blastomeres in each half can compensate for the cell loss and direct normal development.

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**280.** (A) It involves blastomeres flattening and forming tight junctions to establish inner and outer cell layers
Compaction is a critical morphogenetic transition occurring at the late morula stage. Blastomeres maximize intercellular contact, flatten, and form tight junctions. This cellular reorganization polarizes the cells, establishing outer and inner populations. This structural polarization is essential for directing the first lineage differentiation into the trophectoderm and inner cell mass.

**281.** (A) I, II and IV
Somatic cell clones often inherit shortened telomeres from donor cells. While early embryonic reprogramming activates telomerase to elongate telomeres, this restoration can be incomplete. This telomere shortening is associated with premature aging rather than gigantism. Telomerase is the key enzyme responsible for maintaining and elongating telomeres in dividing cells.

**282.** (B) A distinct inner cell mass, a thinned zona pellucida, and a very large fluid-filled cavity
An expanded blastocyst is characterized by a significant increase in the size of the blastocoele cavity, which exerts mechanical pressure on the surrounding shell. This fluid accumulation flattens the trophectoderm cells and stretches the zona pellucida, making it noticeably thinner. The distinct inner cell mass remains clearly visible at one embryonic pole.

**283.** (A) The process is extremely rapid and causes absolutely zero damage or stress to the sperm
Flow cytometry is a slow, highly stressful sorting process that can cause cellular damage to sperm. It requires staining sperm with Hoechst 33342, exposing them to laser excitation, and applying electrostatic charges. This physical stress compromises sperm membrane integrity, which reduces their overall post-sort viability and fertilizing capacity in breeding programs.

**284.** (A) Both A and R are true and R is the correct explanation of A.
In gene pharming, therapeutic transgenes are coupled to mammary-gland specific promoters, such as beta-lactoglobulin. This regulatory design restricts transgene expression strictly to lactating mammary epithelial cells. Confining secretion to the milk prevents the therapeutic protein from entering the animal's circulatory system, protecting its internal physiology from potential bioactive side effects.

**285.** (D) High sperm concentration leading to overwhelming binding before the cortical reaction can successfully complete
The primary cause of polyspermy in standard in vitro fertilization is exposing oocytes to excessively high sperm concentrations. This high sperm-to-oocyte ratio allows multiple sperm to bind and penetrate the zona pellucida simultaneously. This overwhelm occurs before the oocyte can complete cortical granule exocytosis to establish an effective block to polyspermy.

**286.** (A) P-2, Q-3, R-4, S-1
SelF-renewal is the capacity of stem cells to divide continuously while remaining undifferentiated. Differentiation is the developmental process where uncommitted cells become specialized cell types. Pluripotency is the unique ability to give rise to cell types of all three embryonic germ layers. Reprogramming involves reverting mature cells back to a pluripotent state.

NODIA

**287.** (B) III and IV
Follicle-stimulating hormone acts during superovulation to rescue subordinate follicles from atresia and stimulate their growth. However, FSH is not given as a single dose due to its short halF-life; it requires twice-daily injections over several days. Additionally, FSH does not cause luteolysis, which instead requires administering prostaglandin $F_2$ alpha.

**288.** (D) The preovulatory LH surge and subsequent ovulation
Administering a progestin device like a CIDR maintains high systemic progesterone levels in the cow. Progesterone exerts negative feedback on the hypothalamus and anterior pituitary, suppressing the release of gonadotropins. This inhibition strictly prevents the preovulatory luteinizing hormone surge, thereby blocking follicular maturation and ovulation until the device is removed to synchronize the herd.

**289.** (A) I, II, III
Genome editing has evolved toward greater precision and ease of use. Pronuclear microinjection represents the earliest tool, characterized by random transgene integration. Traditional homologous recombination introduced targeted

2017

**19.** Identify the INCORRECT statement regarding capillary electrophoresis.
(A) Separation is driven purely by size exclusion.
(B) It utilizes narrow-bore fused-silica capillaries.
(C) Electro-osmotic flow plays a crucial role in mobilizing all analytes towards the cathode.
(D) High electric fields can be applied due to efficient heat dissipation.

2006

**20.** Which compound is utilized to initiate the polymerization of acrylamide in PAGE?
(A) Bis-acrylamide
(B) N,N,N',N'-tetramethylethylenediamine (TEMED)
(C) Sodium dodecyl sulfate (SDS)
(D) Ammonium persulfate (APS)

2018

**21.** What happens if a reducing agent like beta-mercaptoethanol is omitted during SDS-PAGE of a multimeric protein linked by disulfide bonds?
(A) The protein will fail to enter the resolving gel.
(B) The protein will migrate as a single larger intact complex.
(C) The protein will migrate as multiple separate subunits.
(D) The polyacrylamide matrix will fail to polymerize.

2012

**22.** Which factor directly determines the pore size of a polyacrylamide gel?
(A) The specific pH level of the utilized electrophoresis running buffer.
(B) The starting concentration of the chemical initiators APS and TEMED.
(C) Total acrylamide concentration and the ratio of bis-acrylamide.
(D) The magnitude of the electric voltage gradient applied to the system.

2012

**23.** During alkaline lysis for plasmid isolation, what is the role of Solution II (NaOH + SDS)?
(A) Neutralizes the alkaline environment of the lysate.
(B) Precipitates chromosomal DNA for selective removal.
(C) Resuspends the bacterial pellet in the initial buffer.
(D) Denatures genomic DNA and cellular protein content.

2013

**24.** What is the fundamental basis of plasmid incompatibility?
(A) Sharing the same replication or partitioning mechanisms
(B) Differing antibiotic resistance genes
(C) Variations in plasmid size
(D) Differences in optimal growth temperature

2015

**25.** Consider the following statements regarding CsCl-EtBr density gradient centrifugation.
I. Ethidium bromide intercalates more efficiently into linear DNA than into supercoiled plasmid DNA.
II. Supercoiled plasmid DNA has a higher buoyant density than linear DNA in this gradient.
III. RNA pellets at the bottom of the ultracentrifuge tube.
Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I, II, and III

2016

**26.** Which type of plasmid replication control relies on an RNA-RNA interaction, as seen in $ColE_1$?
(A) Iteron-based control
(B) Antisense RNA-mediated control
(C) Protein-based repressor control
(D) Stringent replication control

2013

**27.** Identify the INCORRECT statement about plasmid biology.
(A) Stringent plasmids are typically maintained at low copy numbers.
(B) Relaxed plasmids require continuous protein synthesis for their replication.
(C) Plasmids utilize the host cell's DNA replication machinery.
(D) Episomes are plasmids capable of integrating into the host chromosome.

2023

**28.** In the context of plasmid isolation, what is the function of potassium acetate in Solution III?
(A) Neutralizes pH and precipitates chromosomal DNA and proteins.
(B) Lyse the bacterial cell wall via enzymatic digestion.
(C) Denatures chromosomal DNA into separate single strands.
(D) Elutes the plasmid DNA from a silica-based

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**299.** (B) IV only
PCR-based embryo sexing involves taking a small biopsy of trophoblast cells, which can slightly reduce viability. The amplification targets male-specific Y-chromosome sequences, such as the SRY gene. However, the presence of an amplification band confirms the embryo is male, not female, as female embryos lack the Y-specific target sequences.

**300.** (C) It must hatch from the zona pellucida
Before a blastocyst can establish physical contact and implant into the maternal uterine endometrium, it must escape from its protective glycoprotein shell. This process is known as hatching, where cellular expansion and enzymatic digestion rupture the zona pellucida. Escaping this shell allows the outer trophectoderm layer to interact directly with the endometrial tissue.

**********

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ligations.

2002

**39.** What is the primary function of Alkaline Phosphatase in molecular cloning?
(A) Adds phosphate groups to the 5' ends of prepared PCR products.
(B) Degrades remaining RNA transcripts within the reaction mixture.
(C) Removes 5' phosphate groups from vectors to prevent selF-ligation.
(D) Synthesizes a complementary DNA strand for double-stranded DNA.

2018

**40.** Which enzyme is utilized to fill in 5' overhangs or remove 3' overhangs to generate blunt ends?
(A) Taq polymerase
(B) Terminal deoxynucleotidyl transferase
(C) Polynucleotide kinase
(D) Klenow fragment of DNA polymerase I

2009

**41.** Identify the INCORRECT pair regarding nucleic acid modifying enzymes.
(A) $S_1$ nuclease - Cleaves double-stranded DNA specifically
(B) Reverse transcriptase - Synthesizes DNA from an RNA template
(C) Polynucleotide kinase - Transfers a phosphate from ATP to the 5' end of DNA
(D) Exonuclease III - Removes nucleotides from the 3' end of double-stranded DNA

2022

**42.** Star activity in restriction endonucleases refers to which phenomenon?
(A) The inability to cut methylated DNA
(B) The relaxation of sequence specificity under suboptimal buffer conditions
(C) Enhanced cleavage efficiency at elevated temperatures
(D) The sequential cleavage of single strands

2017

**43.** Which nomenclature rule applies to the naming of the restriction enzyme EcoRI?
(A) 'E' stands for Endonuclease, 'co' for coli, and 'R' for strain.
(B) Escherichia genus, coli species, RY13 strain, first enzyme isolated.
(C) 'E' stands for Escherichia, 'co' for coli, and 'R' for restriction.
(D) 'Eco' is the genus, 'R' is the species, and 'I' is the order.

2006

**44.** Terminal deoxynucleotidyl transferase (TdT) is distinct from other DNA polymerases because:
(A) It adds nucleotides to the 3' end of DNA without requiring a template.
(B) It possesses 3' to 5' exonuclease activity.
(C) It requires an RNA template.
(D) It is highly thermostable.

2014

**45.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Neoschizomers recognize the same DNA sequence but cleave at different positions.
**Reason (R):** SmaI (cleaves CCC/GGG) and XmaI (cleaves C/CCGGG) are examples of neoschizomers.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2014

**46.** What characteristic makes a bacteriophage lambda vector suitable for genomic libraries over standard plasmids?
(A) It replicates independently of the host cell.
(B) It provides direct antibiotic resistance to the host.
(C) It is easier to isolate via alkaline lysis.
(D) It integrates larger DNA inserts between 20 and 25 kilobases.

2007

**47.** Which vector utilizes the F-factor replicon to maintain a strictly low copy number and stably clone inserts up to 300 kb?
(A) YAC (Yeast Artificial Chromosome)
(B) Cosmid
(C) BAC (Bacterial Artificial Chromosome)
(D) Phagemid

2012

**48.** Identify the key sequences required for a Yeast Artificial Chromosome (YAC) to function properly.
(A) oriC, cos sites, and antibiotic resistance
(B) CEN, TEL, ARS, and a selectable marker
(C) LTRs, gag, pol, and env genes
(D) T-DNA borders and vir genes

NODIA

2004

**49.** What is the defining feature of a cosmid vector?

2024

**9.** Which combination best minimizes the effect of reducing agents in a protein quantitation assay?
(A) Utilizing the BCA assay over the Bradford assay.
(B) Utilizing the Bradford assay over the Lowry assay.
(C) Utilizing the Lowry assay over the UV 280 nm method.
(D) Utilizing Qubit fluorometry for proteins.

2003

**10.** A DNA sample yields an $A_2$60 of 0.5 and an $A_2$80 of 0.25. Which statement is correct?
(A) The total DNA concentration is calculated as 50 μg/mL.
(B) The $A_2$60/280 ratio is 2.0, representing a high purity level.
(C) The sample appears to be heavily contaminated with proteins.
(D) The total DNA concentration is calculated as 12.5 μg/mL.

2016

**11.** Which quantitation method is classified as an end-point binding assay rather than a catalytic reduction assay?
(A) Bradford assay
(B) Lowry assay
(C) Biuret assay
(D) BCA assay

2024

**12.** Which statement correctly identifies a limitation of fluorometric DNA quantitation?
(A) Lacks the fundamental capability to quantify RNA molecules.
(B) Exhibits significantly lower sensitivity than UV spectrophotometry.
(C) Requires standard curves for each specific dye batch and run.
(D) Erroneously overestimates DNA concentration with free nucleotides.

2002

**13.** Which matrix is most appropriate for resolving DNA fragments spanning 50 to 500 base pairs?
(A) 0.5% Agarose
(B) 10% Polyacrylamide
(C) 2% Agarose
(D) Dextran sulfate

2001

**14.** What is the primary function of Sodium Dodecyl Sulfate (SDS) in SDS-PAGE?
(A) Facilitates the cross-linking of the polyacrylamide gel matrix.
(B) Cleaves internal disulfide bonds within multimeric complexes.
(C) Imparts a uniform negative charge-to-mass ratio to proteins.
(D) Serves as a visible tracking dye for monitoring electrophoresis.

2023

**15.** Match the electrophoresis techniques in List I with their primary resolution principle in List II.
List I: (Electrophoresis Technique)
P. SDS-PAGE
Q. Native PAGE
R. Isoelectric focusing
S. Capillary electrophoresis
List II: (Resolution Principle)
1. Charge-to-mass ratio and hydrodynamic size
2. Electro-osmotic flow and electrophoretic mobility
3. Molecular mass alone
4. Isoelectric point (pI)
(A) P-1, Q-3, R-2, S-4
(B) P-3, Q-1, R-4, S-2
(C) P-4, Q-2, R-1, S-3
(D) P-3, Q-4, R-1, S-2

2019

**16.** In 2D-PAGE, what determines the separation of proteins in the second dimension?
(A) Isoelectric point
(B) Hydrophobicity
(C) Tertiary structure
(D) Molecular weight

2018

**17.** Which buffer component is typically used in agarose gel electrophoresis to maintain pH and provide ions for conductivity?
(A) Tris-Acetate-EDTA (TAE)
(B) Sodium chloride-Sodium citrate (SSC)
(C) Phosphate Buffered Saline (PBS)
(D) Tris-HCl (pH 8.0) alone

2014

**18.** Why is polyacrylamide preferred over agarose for separating small nucleic acids?
(A) Offers a non-toxic alternative that is easier for labs to handle.
(B) Accommodates higher voltages without generating excessive heat.
(C) Resolves single-base differences via uniform, consistent pores.
(D) Operates efficiently without the use of chemical cross-linking agents.

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# CHAPTER 07

# Molecular Biology Techniques and Molecular Diagnostics

2023

**1.** At a wavelength of 260 nm, an absorbance of 1.0 in a 1 cm path length cuvette corresponds to which concentration of double-stranded DNA?
(A) 33 µg/mL
(B) 40 µg/mL
(C) 50 µg/mL
(D) 100 µg/mL

2012

**2.** Which of the following best explains why an $A_260/280$ ratio of 1.5 indicates protein contamination in a DNA sample?
(A) Proteins absorb maximally at 260 nm due to aromatic amino acids.
(B) DNA absorbance shifts to 280 nm in the presence of proteins.
(C) Proteins absorb maximally at 280 nm, decreasing the overall ratio.
(D) Contaminating proteins scatter light exclusively at 260 nm.

2016

**3.** What is the primary advantage of utilizing Qubit fluorometry over standard UV spectrophotometry for nucleic acid quantitation?
(A) Prevents contamination overestimation via target-specific dyes.
(B) Relies on the intrinsic UV absorbance of common nucleobases.
(C) Separates and differentiates distinct sizes of DNA fragments.
(D) Monitors peptide bond absorbance at 230 nm for purity checks.

2021

**4.** Identify the INCORRECT statement regarding the BCA protein assay.
(A) Relies on the reduction of $Cu^{2+}$ to $Cu^{1+}$ by proteins in alkaline media.
(B) Measures colorimetric change resulting from a complex formed at 562 nm.
(C) Utilizes bicinchoninic acid to chelate the reduced copper ion effectively.
(D) Exhibits high sensitivity to interference by common detergent reagents.

NODIA

2012

**5.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** The Bradford assay is subject to non-linear standard curves at high protein concentrations.
**Reason (R):** The assay relies on the depletion of free Coomassie Brilliant Blue G-250 dye, leading to a shift from 465 nm to 595 nm.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2024

**6.** Which dye is central to the Lowry protein assay for color development?
(A) Coomassie Brilliant Blue
(B) Ethidium bromide
(C) Folin-Ciocalteu reagent
(D) SYBR Green

2003

**7.** An $A_260/230$ ratio of 1.2 in an isolated RNA sample most likely indicates contamination by which of the following?
(A) Residual aromatic amino acids
(B) Isolated double-stranded DNA
(C) Presence of free nucleotides
(D) Phenol or chaotropic salts

2001

**8.** In the Bradford assay, which amino acid residues primarily interact with the Coomassie dye?
(A) Arginine and aromatic residues
(B) Serine and threonine
(C) Glutamate and aspartate
(D) Cysteine and methionine

column matrix.

2015

**29.** Which molecule facilitates the partitioning of low-copy-number plasmids during cell division?
(A) RepA protein
(B) RNA I
(C) Par operon proteins
(D) Rop protein

2012

**30.** Which characteristic distinguishes a high-copy-number plasmid from a low-copy-number plasmid?
(A) High-copy plasmids usually possess strict partitioning systems.
(B) High-copy plasmids are always selF- transmissible via conjugation.
(C) High-copy plasmids generally exceed 100 kb in physical size.
(D) Relies less on host replication factors such as the protein DnaA.

2018

**31.** Which feature prevents two different plasmids from being stably maintained in the same bacterial cell line without selection pressure?
(A) Presence of multiple antibiotic resistance markers in the cell.
(B) Utilization of different origin of replication sequences in situ.
(C) Shared origin of replication and repressor control mechanism.
(D) Differences in the percentage of GC content between plasmids.

2016

**32.** During CsCl-EtBr ultracentrifugation, what restricts the unwinding of covalently closed circular (ccc) DNA?
(A) Its small molecular weight
(B) The lack of free ends to relieve topological tension
(C) Its association with histones
(D) The high salt concentration

2001

**33.** The addition of which enzyme is essential in the initial resuspension buffer (Solution I) to ensure pure plasmid DNA recovery?
(A) Proteinase K
(B) Lysozyme
(C) DNA Ligase
(D) RNase A

2009

**34.** What is a "replicon" in the context of plasmid biology?
(A) An origin of replication and its associated control elements.
(B) The genetic element controlling conjugative transfer between cells.
(C) The specific genetic region encoding antibiotic resistance genes.
(D) The segment responsible for host cell lysis during infection.

2013

**35.** Consider the following restriction endonucleases.
I. Type I requires ATP and SAM for cleavage.
II. Type II cleaves at specific sites within or adjacent to the recognition sequence.
III. Type III cleaves randomly at large distances from the recognition site.
IV. Type IIS cleaves at a defined distance outside the recognition sequence.
Which of the following is the correct combination of statements?
(A) I and II
(B) II and IV
(C) I, II and IV
(D) I, III and IV

2017

**36.** Which class of restriction enzymes is most widely utilized in standard recombinant DNA cloning protocols?
(A) Type II
(B) Type I
(C) Type III
(D) Type IV

2017

**37.** What defines an "isoschizomer"?
(A) Enzymes that recognize the same sequence but leave different overhangs.
(B) Enzymes that recognize different sequences but produce compatible ends.
(C) Enzymes that restrict activity exclusively to methylated DNA targets.
(D) Different species enzymes recognizing and cutting identical sequences.

2006

**38.** Which statement regarding $T_4$ DNA ligase is correct?
(A) It requires NAD+ as a cofactor.
(B) It requires ATP as a cofactor to catalyze phosphodiester bond formation.
(C) It can only join blunt-ended DNA fragments.
(D) It functions optimally at 37°C for blunt-end

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modifications but suffered from low efficiency. CRISPR-Cas9 is the most modern tool, utilizing RNA guidance to achieve highly efficient, site-specific double-strand breaks.

**290.** (C) Culturing an oocyte until it reaches Metaphase II and extrudes the first polar body
In vitro maturation is the laboratory technique of culturing immature oocytes, harvested at the germinal vesicle stage, to full fertilizability. The process involves incubating these oocytes in specialized maturation media containing gonadotropins and growth factors. Successful maturation is defined by the resumption of meiosis, reaching metaphase II, and extruding the first polar body.

**291.** (A) Both A and R are true and R is the correct explanation of A.
Pre-implantation embryos can tolerate the loss or exclusion of some blastomeres and still develop into viable blastocysts. Early embryonic cells prior to compaction possess high developmental plasticity, allowing them to adjust to cell loss. The remaining healthy blastomeres continue to divide and compensate, forming a functional inner cell mass and trophectoderm.

**292.** (D) ICSI
Intracytoplasmic sperm injection is a micro-assisted fertilization technique that utilizes a micromanipulator to physically deliver a single sperm cell. The operator loads a single spermatozoon into a fine glass injection pipette and pierces both the zona pellucida and oolemma to deposit it directly into the oocyte cytoplasm, bypassing natural fertilization barriers.

**293.** (B) IV only
Reconstructed SCNT embryos require an artificial stimulus to trigger development, often using electrical pulses or chemical ionophores to induce calcium transients. While this activation initiates meiotic and mitotic divisions, it does not guarantee perfect epigenetic reprogramming. Reprogramming remains a major variable, and incomplete resetting of chromatin can cause developmental abnormalities.

**294.** (C) The cumulus oophorus
During natural fertilization, the ovulated oocyte is surrounded by the cumulus oophorus, a dense matrix of granulosa cells embedded in hyaluronic acid. Sperm must navigate and penetrate this outer cellular barrier before they can bind to the zona pellucida. Hyaluronidase enzymes on the sperm membrane facilitate this penetration by digesting the matrix.

**295.** (D) It involves a precise, gradual cooling rate (e.g., 0.3°C to 0.5°C per minute) down to roughly -30°C
Slow-rate embryo freezing protocols utilize a programmable freezer to cool cells at a very precise, gradual rate, typically between 0.3°C and 0.5°C per minute. This controlled rate allows for progressive cellular dehydration, minimizing intracellular ice crystal growth. The straws are cooled down to approximately -30°C before being plunged into liquid nitrogen.

**296.** (A) P-2, Q-1, R-4, S-3
Genomic imprinting is characterized by parent-oF-origin specific gene expression. Epigenetic reprogramming involves resetting a differentiated nucleus to an embryonic state. X-chromosome inactivation serves as a crucial dosage compensation mechanism in female mammals. Somatic cell nuclear transfer is the physical technique of introducing a somatic nucleus into an enucleated recipient oocyte.

**297.** (A) Both A and R are true and R is the correct explanation of A.
Bioreactor animals used in gene pharming are typically generated via SCNT using transfected somatic cells. This is because somatic cells can be selected and verified for stable transgene integration in vitro before cloning. This approach ensures that every resulting live clone carries the transgene, whereas pronuclear microinjection yields highly variable integration rates.

**298.** (B) The capacity to differentiate into all somatic and germ lineages, but not extraembryonic tissues
Pluripotency is the developmental capacity of embryonic stem cells to differentiate into all cell types derived from the three embryonic germ layers: ectoderm, mesoderm, and endoderm. This potential includes forming all somatic tissues and germline cells, but excludes the ability to form extraembryonic tissues, such as the placenta, which distinguishes pluripotency from totipotency.

NODIA

(A) It solely relies on yeast replication machinery for its function.
(B) It is entirely constructed from bacteriophage $M_{13}$ genetic parts.
(C) It transfers DNA via the mechanism of Agrobacterium mediation.
(D) Plasmid containing lambda 'cos' sites for in vitro packaging.

2019
**50.** Which transformation method utilizes short, high-voltage electrical pulses to create transient pores in the cell membrane?
(A) Electroporation
(B) Chemical competence ($CaCl_2$)
(C) Biolistics
(D) Microinjection

2010
**51.** Biolistic gene transfer (gene gun) relies on microcarriers typically composed of which materials?
(A) Iron or magnetic silica
(B) Platinum or silver
(C) Gold or tungsten
(D) Calcium phosphate or DEAE-dextran

2022
**52.** Which mechanism best describes the induction of competence in E. coli using calcium chloride?
(A) Calcium ions shield DNA and LPS negative charges for binding.
(B) It enzymatically degrades the peptidoglycan layer of the cell.
(C) It actively transports DNA through specific channel proteins.
(D) It forces the integration of the plasmid into the host genome.

2010
**53.** A cloning experiment requires the expression of a eukaryotic protein in E. coli. Which type of vector is strictly necessary?
(A) Standard shuttle vector.
(B) Secretion vector with a eukaryotic signal peptide.
(C) Standard YAC vector.
(D) Expression vector with prokaryotic promoter and RBS.

2001
**54.** What limits the maximum size of the insert DNA in standard cosmid cloning?
(A) The efficiency of the restriction enzymes used
(B) The capacity of the lambda phage head during in vitro packaging
(C) The length of the selectable marker gene
(D) The concentration of the ligase enzyme

2019
**55.** Match the vector type in List I with its typical maximum insert capacity in List II.
List I: (Vector Type)
P. Plasmid
Q. Cosmid
R. BAC
S. YAC
List II: (Maximum Insert Capacity)
1. ~15-20 kb
2. ~35-45 kb
3. Up to 300 kb
4. > 1000 kb
(A) P-1, Q-2, R-3, S-4
(B) P-2, Q-1, R-4, S-3
(C) P-1, Q-3, R-2, S-4
(D) P-3, Q-1, R-4, S-2

2023
**56.** Which genetic element allows a shuttle vector to replicate in two disparate host species?
(A) Presence of two distinct selectable markers in the vector sequence.
(B) Utilization of a universal promoter region for gene expression.
(C) Inclusion of polylinker sequences at both ends of the insert site.
(D) Two distinct origins of replication for different host organisms.

2022
**57.** Identify the correct statement regarding phagemid vectors.
(A) They contain both plasmid and filamentous phage origins of replication.
(B) They can exist only as single-stranded DNA in all life cycle stages.
(C) They are exclusively used for RNA interference.
(D) They cannot be infected by helper phages.

2023
**58.** In the blue-white screening technique, what is the role of the inducer IPTG?
(A) Cleaves X-gal directly to produce a blue colored precipitate.
(B) Binds the lac repressor to allow lacZ-alpha peptide expression.
(C) Confers ampicillin resistance directly to the host bacterial cell.
(D) Provides the essential alpha-peptide directly

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the Maxam-Gilbert G-reaction?
(A) I, II, III, IV
(B) II, I, III, IV
(C) III, II, I, IV
(D) II, III, I, IV

2007

**136.** Why has the Maxam-Gilbert method largely been superseded by the Sanger method?
(A) It requires hazardous chemicals and is hard to automate.
(B) Maxam-Gilbert cannot sequence DNA longer than 20 bases.
(C) Maxam-Gilbert is less accurate for GC-rich regions.
(D) Maxam-Gilbert requires RNA templates.

2009

**137.** Which next-generation sequencing (NGS) platform relies on "sequencing by synthesis" utilizing reversible dye-terminators?
(A) Ion Torrent
(B) Illumina
(C) PacBio SMRT
(D) Oxford Nanopore

2007

**138.** How does the Ion Torrent sequencing platform detect nucleotide incorporation?
(A) By capturing fluorescence from a cleaved dye
(B) By detecting the release of a hydrogen ion ($H^+$) which causes a micro-pH change
(C) By measuring changes in electrical current across a protein pore
(D) By detecting light generated by the luciferase enzyme

2009

**139.** Which technology is classified as a single-molecule real-time (SMRT) long-read sequencing method?
(A) Illumina HiSeq
(B) 454 Pyrosequencing
(C) Pacific Biosciences (PacBio)
(D) SOLiD

2003

**140.** Identify the principal mechanism of Oxford Nanopore sequencing.
(A) Sequencing by ligation using fluorophore-labeled probes in situ.
(B) Recording current disruptions as DNA threads through a pore.
(C) Detecting protons released into semiconductor wells in the chip.
(D) Measuring bridge amplification clusters on the flow cell surface.

2019

**141.** What is the primary purpose of "bridge amplification" in the Illumina sequencing workflow?
(A) Generating localized clonal clusters for signal intensity.
(B) To ligate adapters to the DNA fragments in the reaction.
(C) To physically link the DNA polymerase to the flow cell surface.
(D) To repair single-stranded nicks before sequencing begins.

2014

**142.** Which sequencing error is most characteristically associated with homopolymer regions (e.g., AAAAA) in Ion Torrent and Pyrosequencing?
(A) Substitution errors within the sequence string.
(B) Chimeric reads generated during amplification.
(C) Indels resulting from non-linear signal intensity.
(D) Premature termination of the sequencing read.

NODIA

2005

**143.** Which statement accurately compares short-read (Illumina) and long-read (PacBio/Nanopore) technologies?
(A) Short-read offers lower per-base accuracy than early long-read methods.
(B) Long-read is superior for resolving complex genomic repeats and structural variants.
(C) Short-read platforms sequence single unamplified molecules.
(D) Long-read platforms rely exclusively on reversible terminators.

2001

**144.** In PacBio sequencing, where is the DNA polymerase located?
(A) Flowing freely in the sequencing buffer
(B) Immobilized at the bottom of a Zero-Mode Waveguide (ZMW)
(C) Attached to a magnetic bead
(D) Conjugated to the specific fluorescent nucleotides

2005

**145.** Consider the following statements regarding NGS.
I. Illumina produces read lengths typically up

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2019

**68.** Which outcome is expected in colony PCR if vector-specific primers flanking the multiple cloning site are used on a non-recombinant colony?
(A) Amplification of a short fragment from flanking vector DNA.
(B) No amplification occurs at all during the PCR reaction.
(C) Amplification of a large fragment representing the insert size.
(D) Multiple non-specific bands appear on the electrophoresis gel.

2010

**69.** Which method of synthesizing nucleic acid probes utilizes DNase I to create single-stranded gaps followed by DNA polymerase I action?
(A) Random primer labeling
(B) End labeling
(C) In vitro transcription
(D) Nick translation

2024

**70.** What is the primary advantage of random primer labeling over nick translation?
(A) Requires double-stranded intact DNA without prior denaturation.
(B) Generates higher specific activity probes from less template.
(C) It exclusively utilizes RNA polymerase for the labeling process.
(D) It inherently produces non-radioactive probes for detection.

2017

**71.** Which enzyme is explicitly required for 5' end labeling of a dephosphorylated DNA probe using [gamma-32P] ATP?
(A) Terminal deoxynucleotidyl transferase
(B) Klenow fragment
(C) $T_4$ Polynucleotide kinase
(D) Reverse transcriptase

2008

**72.** Identify the INCORRECT statement comparing radioactive and non-radioactive probes.
(A) Radioactive probes generally offer higher sensitivity for single-copy gene detection.
(B) Non-radioactive probes (e.g., biotinylated) have longer shelf lives.
(C) Non-radioactive probes emit higher radiation than P-32 probes.
(D) Non-radioactive probes require stringent isotope disposal protocols.

2014

**73.** Which molecule is commonly incorporated into nucleotides for non-radioactive probe labeling and is detected utilizing an avidin-conjugated enzyme?
(A) Biotin
(B) Digoxigenin
(C) Fluorescein
(D) Cyanine 5

2015

**74.** How does the Klenow fragment differ from holoenzyme DNA polymerase I during random primer labeling?
(A) It possesses enhanced 5' to 3' exonuclease activity in situ.
(B) Lacks 5'-3' exonuclease to prevent new probe degradation.
(C) It is entirely independent of a template strand for synthesis.
(D) It utilizes RNA primers exclusively for the labeling reaction.

2004

**75.** Consider the following labeling methods.
I. Nick translation requires DNase I.
II. Random primer labeling utilizes hexanucleotides.
III. 3' end labeling utilizes Terminal deoxynucleotidyl transferase.
IV. 5' end labeling utilizes alkaline phosphatase and $T_4$ DNA ligase.
Which combination identifies the correct statements?
(A) I, II and III
(B) II, III and IV
(C) I, III and IV
(D) I, II and IV

NODIA

2021

**76.** What characterizes digoxigenin (DIG) labeled probes in molecular diagnostics?
(A) Detection via anti-DIG antibodies coupled to reporter enzymes.
(B) They emit intrinsic fluorescence upon hybridization to targets.
(C) They are highly radioactive isotopes of the hydrogen atom.
(D) They require photographic emulsion for signal detection steps.

2003

**77.** Which sequence feature is essential when designing synthetic oligonucleotide probes to ensure specific hybridization?
(A) High poly-A content to prevent secondary

structures in probes.
(B) Palindromic sequences to allow selF-annealing of the molecule.
(C) Sufficient length (18-30 bases) and appropriate GC content.
(D) Repetitive sequence motifs to enhance signal strength in assays.

2025
**78.** In the context of Green Fluorescent Protein (GFP), which amino acid residues undergo cyclization and oxidation to form the mature chromophore?
(A) His65-Tyr66-Gly67
(B) Tyr65-Trp66-Gly67
(C) Ser65-Phe66-Gly67
(D) Ser65-Tyr66-Gly67

2012
**79.** What is a strict requirement for the maturation of the wild-type GFP chromophore?
(A) The presence of a heavy metal cofactor
(B) Hydrolysis of ATP
(C) Molecular oxygen ($O_2$)
(D) An external light source during folding

2011
**80.** How do engineered variants of GFP, such as EGFP, primarily differ from the wild-type protein?
(A) They require a distinct set of cofactors for fluorescence activity.
(B) Point mutations shift excitation peaks and increase intensity.
(C) They are significantly smaller in overall molecular weight.
(D) They lose the ability to form a beta-barrel three-dimensional structure.

2008
**81.** Which structural motif characterizes the overall three-dimensional shape of GFP?
(A) Alpha-helical bundle
(B) Zinc finger
(C) Leucine zipper
(D) Beta-barrel

2006
**82.** Which application is LEAST suited for GFP as a reporter gene?
(A) Quantifying precise enzymatic reaction kinetics in vitro
(B) Live-cell imaging of protein localization
(C) Real-time monitoring of gene expression
(D) Tracking temporal dynamics of viral infection

2007
**83.** Why is GFP often fused to the N- or C-terminus of a target protein in cell biology?
(A) To induce the targeted degradation of the protein in the cell.
(B) To alter the target protein's enzymatic activity levels in situ.
(C) Non-toxic visible tag for tracking subcellular localization.
(D) To ensure the protein is secreted from the cell into the media.

2015
**84.** Which phenomenon enables the generation of differently colored fluorescent proteins (e.g., YFP, BFP) from the original GFP sequence?
(A) Amino acid substitutions altering the chromophore environment.
(B) Splicing of the mRNA transcript during gene expression.
(C) Conjugation with synthetic fluorophores in a live cell environment.
(D) Varying the concentration of molecular oxygen in the system.

2002
**85.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** GFP fluorescence can be used to study dynamic protein-protein interactions via FRET.
**Reason (R):** The emission spectrum of a donor GFP variant can overlap with the excitation spectrum of an acceptor variant.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2006
**86.** Which limitation is associated with the use of standard GFP in deep tissue in vivo imaging?
(A) The beta-barrel structure unravels at mammalian temperatures.
(B) Tissue absorbance and scattering of emission wavelengths.
(C) It is highly toxic to mammalian cells during long-term imaging.
(D) It requires exogenous substrate addition prior to imaging steps.

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2003

**87.** A researcher wants to visualize the simultaneous expression of two different genes in the same cell. What is the most appropriate approach?
(A) Use wild-type GFP for both, but image at different times.
(B) Use X-gal staining for both genes in the cell population.
(C) Use spectral variants like EGFP and mCherry for distinction.
(D) Use radioactive probes targeting both mRNAs in the sample.

2008

**88.** The melting temperature ($T_m$) of a DNA duplex is defined as:
(A) The temperature where the DNA molecule is fully single-stranded.
(B) The temperature at which the DNA begins to anneal in solution.
(C) The specific temperature required to break phosphodiester bonds.
(D) Temperature where 50% of the DNA duplexes are dissociated.

2024

**89.** Which base composition directly increases the melting temperature ($T_m$) of a DNA duplex?
(A) High proportion of Guanine and Cytosine
(B) High proportion of Adenine and Thymine
(C) High proportion of Uracil
(D) Equal proportions of all four bases

2012

**90.** In nucleic acid hybridization, what effect does decreasing the monovalent salt concentration have on the stringency of the reaction?
(A) Decreases stringency by stabilizing mismatched duplexes in situ.
(B) Increases stringency via electrostatic repulsion of backbones.
(C) It has no measurable effect on the stringency of the reaction.
(D) It irreversibly denatures all probes utilized in the protocol.

2009

**91.** Which formula provides a simple approximation of $T_m$ for short oligonucleotides (less than 20 bases)?
(A) $T_m = 2(G+C)+4(A+T)$
(B) $T_m = 4(G+C)+2(A+T)$
(C) $T_m = 81.5 + 16.6\log[M] + 0.41(\%GC)$
(D) $T_m = \frac{A+T}{G+C}$

2016

**92.** Cot curve analysis measures the rate of DNA reassociation. What does a low $\text{Cot}_{\frac{1}{2}}$ value indicate about a specific DNA fraction?
(A) It represents highly repetitive DNA sequences that find their partners quickly.
(B) It represents highly complex, unique single-copy sequences.
(C) It represents RNA molecules rather than DNA.
(D) It represents DNA with an exceptionally high GC content.

2016

**93.** What is the primary purpose of adding formamide to a hybridization buffer?
(A) To increase the rate of DNA degradation in the reaction mix.
(B) To act as a non-specific blocking agent for the membrane.
(C) Lowers $T_m$ to allow hybridization at reduced temperatures.
(D) To increase the background fluorescence during detection steps.

NODIA

2021

**94.** Identify the INCORRECT statement regarding hybridization stringency.
(A) High temperature increases stringency.
(B) Low salt concentration increases stringency.
(C) Formamide addition increases stringency at a given temperature.
(D) High stringency allows multiple base-pair probe mismatches.

2016

**95.** Match the hybridization parameter in List I with its effect on DNA duplex stability in List II.
List I: (Hybridization Parameter)
P. High GC content
Q. High salt ($Na^+$) concentration
R. Presence of formamide
S. Mismatched base pairs
List II: (Effect on Stability)
1. Decreases $T_m$ by interfering with hydrogen bonds
2. Decreases stability by disrupting helix geometry
3. Increases $T_m$ due to three hydrogen bonds
4. Increases $T_m$ by shielding phosphate repulsion
(A) P-4, Q-3, R-2, S-1
(B) P-3, Q-4, R-1, S-2
(C) P-3, Q-1, R-4, S-2
(D) P-1, Q-4, R-3, S-2

to the bacteria.

2014

**59.** What structural mechanism enables insertional inactivation during recombinant screening?
(A) The vector's origin of replication is deleted during cloning.
(B) The foreign DNA is inserted into a selectable marker gene, disrupting its function.
(C) The restriction enzyme digests the host chromosome.
(D) The inserted gene secretes an inactivating enzyme.

2001

**60.** Alpha-complementation occurs when:
(A) Plasmid alpha-peptide and host omega-peptide form beta-gal.
(B) A full-length lacZ gene on a plasmid is expressed in the cell.
(C) The inserted DNA fragments complement host metabolic deficiency.
(D) X-gal molecules directly bind to the inserted DNA sequence.

2018

**61.** Why do recombinant colonies appear white in blue-white screening?
(A) The insert DNA produces a bleaching agent in the cell.
(B) The recombinant cells fail to absorb X-gal from the agar.
(C) Disrupted lacZ-alpha prevents X-gal cleavage by beta-gal.
(D) The insert DNA suppresses the antibiotic resistance gene.

2005

**62.** Which method is most efficient for rapidly verifying the presence and size of an insert directly from cultured bacterial colonies without plasmid isolation?
(A) Southern blotting
(B) Next-generation sequencing
(C) Mass spectrometry
(D) Colony PCR

2004

**63.** In colony PCR, what serves as the initial source of template DNA?
(A) Purified plasmid DNA extracted via standard alkaline lysis.
(B) Isolated chromosomal DNA from the host bacterial organism.
(C) Bacterial cells lysed during the initial denaturation step.
(D) RNA transcribed by the colony during its growth phase.

2021

**64.** A researcher uses a vector with an intact tetracycline resistance gene and a disrupted ampicillin resistance gene (due to cloning). Which phenotype indicates a successful recombinant?
(A) Ampicillin resistant, Tetracycline sensitive
(B) Ampicillin sensitive, Tetracycline resistant
(C) Sensitive to both antibiotics
(D) Resistant to both antibiotics

2010

**65.** Match the screening component in List I with its biochemical function in List II.
List I: (Screening Component)
P. X-gal
Q. IPTG
R. Beta-galactosidase
S. Lac repressor
List II: (Biochemical Function)
1. Cleaves lactose analogs
2. Binds operator to prevent transcription
3. Chromogenic substrate
4. Induces transcription by binding repressor
(A) P-1, Q-3, R-2, S-4
(B) P-3, Q-1, R-4, S-2
(C) P-4, Q-3, R-2, S-1
(D) P-3, Q-4, R-1, S-2

2019

**66.** Which false positive scenario can occur in blue-white screening?
(A) Small in-frame insertions that do not abolish enzyme activity.
(B) Complete loss of the plasmid, resulting in blue colonies.
(C) Overexpression of the lacZ gene, turning the media red.
(D) Resistance to IPTG, resulting in white colonies.

2004

**67.** What is the fundamental requirement for the host E. coli strain used in alpha-complementation?
(A) It must completely lack the lacZ gene sequence in its genome.
(B) It must possess a naturally high resistance to ampicillin drug.
(C) It must carry a lacZ mutation (e.g. $\Delta M_{15}$) deleting the alpha part.
(D) It must naturally express the blue chromophore in its cytosol.

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2022
**106.** In an ISH protocol, what is the primary role of Proteinase K treatment?
(A) Digesting proteins to facilitate probe access to nucleic acids.
(B) Degrading target mRNA molecules within the tissue sample.
(C) Acting as the detection enzyme for colorimetric assay results.
(D) Neutralizing the fixative chemicals used in tissue preparation.

2017
**107.** Which advantage does FISH possesses over traditional colorimetric ISH?
(A) It requires less complex microscopy equipment for visualization.
(B) Enables multiplexing via spectrally distinct fluorophore labels.
(C) The signals are permanent and never experience photobleaching.
(D) It does not require sequence-specific probes for hybridization.

2022
**108.** A HER2 FISH assay on breast cancer tissue typically utilizes a probe for the HER2 gene and a control probe for which chromosomal structure?
(A) The telomere region of human chromosome 1.
(B) Centromere of chromosome 17 (CEP17) control probe.
(C) The ribosomal RNA gene cluster of the host cell.
(D) The mitochondrial DNA within the breast cancer cell.

2017
**109.** What defines "Fiber-FISH" compared to interphase or metaphase FISH?
(A) Maps genes on extended single DNA fibers for high resolution.
(B) It utilizes probes attached to specialized optical fibers.
(C) It targets RNA molecules exclusively for visualization.
(D) It visualizes whole intact organisms during the imaging step.

2019
**110.** Which physical process is responsible for generating an image in standard autoradiography?
(A) Emission of visible light stimulating a photomultiplier tube.
(B) Silver halide reduction in emulsion by ionizing radiation.
(C) Excitation of a fluorophore by a laser during detection.
(D) Measurement of mass-to-charge ratios of radioactive ions.

2019
**111.** What is the approximate biological halF-life of Phosphorus-32 (32P)?
(A) 8 days
(B) 14.3 days
(C) 60 days
(D) 5730 years

2015
**112.** Which radioisotope is most commonly used for labeling proteins via methionine incorporation?
(A) 32P
(B) 14C
(C) 35S
(D) 3H

2010
**113.** Tritium (3H) is a low-energy beta emitter. What is its approximate halF-life?
(A) 14.3 days
(B) 87.4 days
(C) 12.3 years
(D) 5730 years

NODIA

2021
**114.** Identify the correct ranking of beta-emission energy (from strongest to weakest) for these common radioisotopes.
(A) $32P > 14C > 35S > 3H$
(B) $32P > 35S > 14C > 3H$
(C) $3H > 14C > 35S > 32P$
(D) $35S > 32P > 3H > 14C$

2018
**115.** Match the radioisotope in List I with its typical molecular biology application in List II.
List I: (Radioisotope)
P. 32P
Q. 35S
R. 3H
S. 14C
List II: (Application)
1. Labeling amino acids in pulse-chase experiments
2. End-labeling of DNA probes
3. High-resolution whole-body autoradiography
4. Long-term metabolic tracing and dating
(A) P-2, Q-1, R-3, S-4
(B) P-1, Q-2, R-4, S-3
(C) P-2, Q-3, R-1, S-4

2021

**96.** During a Cot curve experiment, the genomic DNA of a eukaryotic organism typically displays three distinct phases. Which fraction reassociates last?
(A) Highly repetitive DNA
(B) Single-copy (unique) DNA
(C) Moderately repetitive DNA
(D) Satellite DNA

2004

**97.** The term "hyperchromic shift" in DNA thermodynamics refers to:
(A) The shift of absorbance from 260 nm to 280 nm upon melting.
(B) The color change observed when DNA intercalating dyes bind.
(C) The emission of fluorescence upon heating of the DNA sample.
(D) The increase in 260 nm UV absorbance during DNA denaturation.

2024

**98.** Which application relies most critically on precise calculation of oligonucleotide $T_m$?
(A) PCR primer design
(B) Northern blotting
(C) SDS-PAGE
(D) Plasmid miniprep

2016

**99.** Which of the following describes the effect of urea or formamide on the stability of a DNA duplex during hybridization?
(A) Increases the melting temperature ($T_m$) by strengthening hydrogen bonds.
(B) Decreases the melting temperature ($T_m$) by disrupting hydrogen bonds.
(C) Stabilizes the duplex against high temperature denaturation.
(D) Has no effect on the melting temperature ($T_m$) of the duplex.

2019

**100.** In Fluorescence In Situ Hybridization (FISH), what serves to prevent non-specific binding of the fluorescent probe?
(A) Cot-1 DNA or blocking reagents
(B) Ethidium bromide
(C) Formaldehyde
(D) DAPI

2013

**101.** What is the fundamental diagnostic use of chromosome painting in clinical cytogenetics?
(A) Detecting single nucleotide polymorphisms (SNPs) in the genome.
(B) Identifying chromosomal translocations and structural rearrangements.
(C) Quantifying exact mRNA expression levels in a cell population.
(D) Determining the precise sequence of a single target gene.

2005

**102.** Which counterstain is most frequently used in FISH to visualize the overall nuclear morphology?
(A) Coomassie Blue
(B) SYBR Green
(C) Crystal Violet
(D) DAPI (4',6-diamidino-2-phenylindole)

2017

**103.** Identify the INCORRECT statement regarding FISH methodology.
(A) Probes are typically labeled with fluorophores directly or via haptens.
(B) Cells or tissues must be fixed and permeabilized prior to hybridization.
(C) A fluorescence microscope is required for signal detection.
(D) Target DNA must stay double-stranded during the entire protocol.

2023

**104.** Which variant of FISH is designed to paint the entire karyotype of an organism using multiple fluorochromes simultaneously?
(A) Fiber-FISH resolution imaging.
(B) Q-FISH quantitative imaging.
(C) Spectral Karyotyping (SKY) or Multiplex FISH (M-FISH).
(D) Flow-FISH cell analysis.

2004

**105.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** ISH can be used to detect viral RNA within host tissue biopsies.
**Reason (R):** ISH probes can be designed to specifically hybridize to viral transcripts while preserving cellular architecture.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

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binding sites on the membrane.

Which of the above statement(s) is/are correct?

(A) I and II
(B) II and III
(C) I and III
(D) I, II, and III

2008

**126.** What is the purpose of baking (80°C) or UV irradiation of a nylon membrane after DNA transfer?

(A) To visualize the DNA directly
(B) To covalently crosslink the nucleic acids to the membrane matrix
(C) To melt the remaining agarose fragments
(D) To neutralize the alkaline transfer buffer

2018

**127.** Which probe type is predominantly used to detect the target in a Northern blot?

(A) A labeled antibody
(B) A specific labeled single-stranded DNA or RNA sequence
(C) A fluorescent intercalating dye
(D) A heavy metal isotope

2005

**128.** What constitutes the defining chemical distinction between ribo- and deoxyribonucleotides utilized in Sanger sequencing?

(A) Dideoxynucleotides (ddNTPs) lack a 2'-hydroxyl group.
(B) Dideoxynucleotides contain an extra phosphate group.
(C) Dideoxynucleotides (ddNTPs) lack a 3'-hydroxyl group.
(D) Dideoxynucleotides contain a modified nitrogenous base.

2025

**129.** How does the incorporation of a ddNTP terminate DNA synthesis in the Sanger method?

(A) Missing 3'-OH prevents further phosphodiester bond formation.
(B) It permanently denatures the DNA polymerase enzyme in situ.
(C) It triggers the 3' to 5' exonuclease activity of the polymerase.
(D) It introduces a bulky fluorescent moiety sterically hindering growth.

2005

**130.** The Maxam-Gilbert sequencing method is primarily based on which principle?

(A) Enzymatic chain termination using specific polymerase inhibitors
(B) Base-specific chemical modification and subsequent cleavage of the DNA backbone
(C) Detection of pyrophosphate release during nucleotide incorporation
(D) Reversible dye-terminators

2015

**131.** In the original Maxam-Gilbert protocol, which chemical is utilized to specifically modify guanine residues?

(A) Hydrazine
(B) Dimethyl sulfate (DMS)
(C) Piperidine
(D) Formic acid

NODIA

2005

**132.** Identify the INCORRECT feature of automated Sanger sequencing using fluorescent dyes.

(A) It utilizes four distinct fluorescent dyes, one for each ddNTP.
(B) All four sequencing reactions (A, C, G, T) can be multiplexed in a single capillary tube.
(C) An electropherogram is generated based on emission peaks over time.
(D) Sequence is read via measuring pyrophosphate luminescence.

2005

**133.** What dictates the ratio of dNTPs to ddNTPs in a Sanger sequencing reaction?

(A) Requirement to generate fragments terminating at every position.
(B) The requirement to entirely inhibit normal DNA synthesis in situ.
(C) The buffering capacity of the utilized sequencing reaction mix.
(D) The size of the template DNA molecule being sequenced.

2010

**134.** Which enzyme was classically modified (e.g., Sequenase) to lack 3'-5' exonuclease activity for optimal Sanger sequencing?

(A) Taq polymerase
(B) $T_4$ DNA ligase
(C) $T_7$ DNA polymerase
(D) Reverse transcriptase

2012

**135.** Consider the following sequencing steps.

I. Piperidine cleavage
II. Dimethyl sulfate treatment
III. Separation on a high-resolution polyacrylamide gel
IV. Autoradiography

Which of the following is the correct order for

(D) P-3, Q-1, R-2, S-4

2024

**116.** Why are intensifying screens frequently used with 32P in autoradiography at -70°C?
(A) To slow down the radioactive decay of the isotope significantly.
(B) Converts beta particles to light to increase detection sensitivity.
(C) To prevent the film from freezing during the exposure process.
(D) To absorb background cosmic radiation within the laboratory.

2023

**117.** Which statement accurately describes a limitation of using 3H in standard X-ray film autoradiography?
(A) Its halF-life is too short for practical use in most labs.
(B) Its high-energy emission overexposes the film instantly.
(C) It chemically reacts with the silver halide in the emulsion.
(D) Weak beta particles cannot penetrate film coating effectively.

2019

**118.** Which safety measure is explicitly recommended for shielding the high-energy beta emissions of 32P?
(A) Lead aprons and thick lead glass
(B) Thin aluminum foil
(C) Thick Plexiglas or acrylic shielding
(D) Heavy water

2021

**119.** In photographic emulsion chemistry, what is formed when ionizing radiation strikes a silver bromide crystal?
(A) A latent image consisting of metallic silver atoms in the crystal.
(B) A fluorescent photon emitted from the crystal lattice.
(C) A colored precipitate of formazan on the film surface.
(D) A secondary electron cascade through the emulsion layer.

2017

**120.** Which technique detects specific DNA fragments separated by gel electrophoresis through hybridization with a labeled probe?
(A) Southern blotting
(B) Northern blotting
(C) Western blotting
(D) Eastern blotting

2015

**121.** What is the fundamental difference in the target molecule between Northern and Western blotting?
(A) Northern detects DNA; Western detects RNA.
(B) Northern detects proteins; Western detects DNA.
(C) Northern detects RNA; Western detects proteins.
(D) Northern detects lipids; Western detects carbohydrates.

2019

**122.** In Southern blotting, why is the agarose gel often treated with a dilute acid (e.g., HCl) prior to alkaline transfer?
(A) To neutralize the running buffer within the agarose matrix.
(B) To denature the DNA into single strands for hybridization.
(C) Depurinates DNA to fragment it for efficient membrane transfer.
(D) To permanently crosslink DNA to the gel for stable transfer.

2014

**123.** Which macromolecule does Southwestern blotting specifically analyze?
(A) RNA-protein interactions
(B) DNA-binding proteins
(C) Protein-protein interactions
(D) Glycoproteins

2022

**124.** Identify the INCORRECT statement concerning membrane supports used in blotting.
(A) Nitrocellulose membranes are highly fragile when dry.
(B) Nylon membranes possess greater mechanical strength than nitrocellulose.
(C) PVDF membranes require pre-wetting in methanol prior to use.
(D) Nitrocellulose binds DNA covalently without baking or UV.

2013

**125.** Consider the following statements about Western blotting.
I. Primary antibodies bind directly to the target protein.
II. Secondary antibodies are conjugated to a reporter enzyme like HRP.
III. Milk or BSA is used to block non-specific

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to 300 bp.
II. Nanopore sequencing does not require DNA amplification.
III. Ion Torrent utilizes fluorescently labeled dNTPs.
IV. PacBio uses Zero-Mode Waveguides to restrict the observation volume.

Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, III and IV
(C) II, III and IV
(D) I, II and III

2023
**146.** Which fundamental property governs the separation of proteins in ion-exchange chromatography?
(A) Electrostatic interaction between charges and stationary phase.
(B) The total molecular weight of the protein analyte.
(C) Hydrophobicity levels of the protein surface residues.
(D) Specific biological affinity for the column ligand.

2024
**147.** A protein with a high isoelectric point ($pI = 9.5$) is dissolved in a buffer at pH 7.0. Which type of chromatographic resin will it bind to most effectively?
(A) Cation-exchange resin
(B) Anion-exchange resin
(C) Size-exclusion resin
(D) Hydrophobic interaction resin

2012
**148.** In gel filtration (size exclusion) chromatography, which molecules elute first?
(A) Smallest molecules as they enter matrix pores and delay.
(B) Largest molecules as they are excluded into void volume.
(C) Positively charged molecules within the mobile phase.
(D) Molecules with the highest affinity for the column matrix.

2007
**149.** Which affinity tag specifically binds to immobilized metal ions (e.g., $Ni^{2+}$ or $CO_2^{+}$) via coordinate covalent bonds?
(A) GST-tag
(B) His-tag
(C) FLAG-tag
(D) MBP-tag

2002
**150.** How is a His-tagged protein typically eluted from a Nickel-NTA affinity column?
(A) By washing with a high salt concentration buffer
(B) By washing with a buffer containing a high concentration of imidazole
(C) By changing the temperature
(D) By adding reducing agents like DTT

2025
**151.** Identify the INCORRECT pairing of affinity tag and its elution agent.
(A) GST-tag - Reduced Glutathione
(B) Maltose Binding Protein (MBP) - Maltose
(C) His-tag - Imidazole
(D) Protein A - High pH buffer (e.g., pH 10.0)

2023
**152.** Which mechanism explains the resolution of proteins in an anion-exchange chromatography column using a salt gradient?
(A) Salt ions progressively alter the protein's folded structure.
(B) Salt ions compete with negative proteins for resin sites.
(C) Salt causes proteins to precipitate and elute from the column.
(D) Salt changes the pore size of the resin within the column.

2019
**153.** What is the primary purpose of adding a GST-tag to a recombinant protein beyond purification?
(A) Enhances solubility of the expressed fusion protein.
(B) It acts as a fluorescent reporter for tracking.
(C) It ensures secretion into the culture medium.
(D) It provides intrinsic protease activity to the protein.

2009
**154.** Restriction Fragment Length Polymorphism (RFLP) analysis fundamentally relies on which molecular tools?
(A) Reverse transcriptase and specific primers
(B) Restriction endonucleases and sequence-specific hybridization probes
(C) RNA polymerase and fluorescent nucleotides
(D) Mass spectrometry

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2024
**193.** Which application is an array-CGH (Comparative Genomic Hybridization) specifically designed for?
(A) Measuring relative mRNA expression levels in tissues.
(B) Detecting chromosomal copy number deletions and duplications.
(C) Determining the three-dimensional structure of chromosomes.
(D) Sequencing the entire genome rapidly in a single step.

2018
**194.** Why is target RNA typically converted to cDNA or cRNA prior to labeling and microarray hybridization?
(A) RNA is too bulky to hybridize to surface probes in the chip.
(B) RNA is highly unstable and prone to degradation during the protocol.
(C) Fluorescent dyes cannot chemically bind to ribose sugars in RNA.
(D) Arrays only contain RNA probes for hybridization detection.

2008
**195.** What is the functional role of the matrix in Matrix-Assisted Laser Desorption/Ionization (MALDI)?
(A) To physically separate peptides based on size within the matrix.
(B) Absorbs laser energy to facilitate soft analyte ionization.
(C) To digest the proteins into specific peptides for analysis.
(D) To act as a vacuum seal for the mass spectrometer interface.

2018
**196.** Which statement correctly distinguishes Electrospray Ionization (ESI) from MALDI?
(A) ESI is a hard ionization technique causing extensive fragmentation; MALDI is soft.
(B) ESI generates multiply charged liquid ions; MALDI generates solid singly charged ions.
(C) ESI requires a pulsed laser; MALDI requires a high-voltage capillary.
(D) ESI is exclusively coupled with Time-oF-Flight (TOF) analyzers.

2012
**197.** In Peptide Mass Fingerprinting (PMF), what is the first critical step before mass spectrometry?
(A) Sequence-specific cleavage of the purified protein into peptides using an enzyme like Trypsin
(B) Complete hydrolysis of the protein into individual amino acids
(C) Direct injection of the intact protein into the MS/MS
(D) Conjugation of the protein with a fluorescent dye

2011
**198.** How does a Time-oF-Flight (TOF) mass analyzer determine the mass-to-charge ($\frac{m}{z}$) ratio of an ion?
(A) By recording the time it takes for an accelerated ion to travel a fixed distance through a field-free drift tube
(B) By measuring the magnetic field required to deflect the ion
(C) By trapping the ion in a radio-frequency field
(D) By measuring the ion's absorbance of laser light

2001
**199.** Tandem Mass Spectrometry (MS/MS) is essential for which of the following proteomics tasks?
(A) Determining the overall shape of a protein complex in situ.
(B) Obtaining de novo peptide sequence via precursor fragmentation.
(C) Measuring the precise concentration of a protein without standards.
(D) Synthesizing novel peptides within the mass spectrometer.

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2013
**200.** Identify the INCORRECT statement regarding mass spectrometry-based proteomics.
(A) Mass spectrometry directly sequences the mRNA corresponding to the protein.
(B) Trypsin cleaves specifically at the C-terminal side of lysine and arginine residues.
(C) MALDI is frequently coupled with TOF mass analyzers.
(D) MS/MS utilizes an inert gas to fragment peptides along the peptide backbone.

2016
**201.** Which component of a biosensor is responsible for converting a biological interaction into a measurable electrical signal?
(A) The transducer
(B) The bioreceptor (e.g., antibody or enzyme)
(C) The semi-permeable membrane
(D) The target analyte

List I: (Molecular Marker)
P. SNP
Q. SSR
R. RFLP
S. AFLP
List II: (Analytical Principle)
1. Single base pair variation detection
2. Length variation of short tandem repeats
3. Variation in restriction fragment lengths via hybridization
4. PCR amplification of restriction fragments with adapter-specific primers
(A) P-2, Q-1, R-4, S-3
(B) P-1, Q-2, R-3, S-4
(C) P-1, Q-3, R-2, S-4
(D) P-3, Q-1, R-4, S-2

2025
**166.** Which technique is most commonly employed in modern forensic DNA profiling due to its high sensitivity and discriminatory power?
(A) RFLP analysis of VNTRs via Southern blotting
(B) Whole-genome Maxam-Gilbert sequencing
(C) PCR multiplexing of highly polymorphic STR (microsatellite) loci
(D) RAPD profiling

2008
**167.** Identify the INCORRECT statement regarding Single Nucleotide Polymorphisms (SNPs).
(A) They represent the most abundant form of genetic variation in the human genome.
(B) They are typically multi-allelic, with up to four possible alleles per locus common in populations.
(C) They can be detected using microarrays or next-generation sequencing.
(D) They can occur in both coding and non-coding regions.

2024
**168.** In forensic DNA analysis, what does the term "allelic ladder" refer to?
(A) A chemical gradient used to separate DNA
(B) A mixture of common alleles for specific STR loci used as a size reference standard
(C) The physical structure of the polyacrylamide gel
(D) The hierarchy of dominant and recessive genes

2008
**169.** What is a "null allele" in the context of microsatellite (SSR) genotyping?
(A) An allele that produces a toxic protein
(B) An allele that fails to amplify during PCR due to a mutation in the primer binding site
(C) An allele entirely devoid of tandem repeats
(D) An allele that has integrated into a different chromosome

2004
**170.** Which component is NOT absolutely essential for a standard Polymerase Chain Reaction (PCR)?
(A) Thermostable DNA polymerase
(B) Deoxynucleotide triphosphates (dNTPs)
(C) RNA primase
(D) Sequence-specific DNA primers

2013
**171.** During the thermal cycling kinetics of PCR, what primarily occurs at 94-95°C?
(A) Primers anneal to the template.
(B) The double-stranded DNA template denatures into single strands.
(C) The Taq polymerase synthesizes the new strand at its optimal rate.
(D) The phosphodiester backbone is cleaved.

2022
**172.** The annealing temperature in a PCR protocol is typically set based on which parameter?
(A) The extension rate of the polymerase
(B) The size of the expected amplicon
(C) The concentration of magnesium chloride
(D) The melting temperature (Tm) of the primers

2003
**173.** What is the primary advantage of Nested PCR over standard PCR?
(A) Reduces the total reaction time by half.
(B) Significantly increases specificity and sensitivity of amplification.
(C) Allows direct sequencing of products without purification.
(D) Amplifies RNA molecules directly from the sample.

2011
**174.** Multiplex PCR is characterized by the use of:
(A) Single primer pairs designed to amplify multiple different templates.
(B) Multiple distinct primer pairs in a single reaction tube.
(C) Successive rounds of PCR using the same primer set.
(D) Primers labeled with radioactive isotopes for detection.

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without affecting RNA.

2013

**230.** During the preparation of competent E. coli cells using the rubidium chloride method, why must the cells be kept continuously on ice?
(A) Hardens the lipid bilayer to stabilize the competent state.
(B) To prevent the cells from rapidly dividing and losing the plasmid.
(C) To irreversibly denature external nucleases in the environment.
(D) To precipitate the rubidium salts into the cell cytoplasm.

2001

**231.** Which feature defines a Type IIS restriction endonuclease (e.g., FokI)?
(A) It requires dual recognition sites oriented in opposite directions.
(B) It cleaves at a precise, defined distance outside of its asymmetric recognition sequence.
(C) It solely functions as a methyltransferase.
(D) It only cleaves single-stranded DNA.

2004

**232.** What is the primary advantage of cloning with Type IIS restriction enzymes in techniques like Golden Gate assembly?
(A) Removes recognition sites for scarless, seamless assembly.
(B) They produce blunt ends exclusively for ligation reactions.
(C) They do not require ATP or magnesium for DNA cleavage.
(D) They cut randomly, producing a highly diverse genomic library.

2014

**233.** In blue-white screening, a colony appears pale blue instead of distinct white or dark blue. What is the most plausible molecular explanation?
(A) The cloning vector entirely lacks the lacZ gene sequence.
(B) In-frame insertion that altered but did not abolish activity.
(C) The insert contains an in-frame functional beta-galactosidase gene.
(D) The agar plates lacked the antibiotic ampicillin during growth.

2021

**234.** Consider the process of preparing a genomic library.
I. Partial digestion of genomic DNA
II. Ligation into a suitable vector
III. Packaging into phage heads
IV. Size fractionation of DNA fragments
Which of the above sequence is correct?
(A) I, II, IV, III
(B) I, IV, II, III
(C) IV, I, II, III
(D) I, IV, III, II

2007

**235.** Why is "partial digestion" crucial when constructing a representative genomic library?
(A) Complete digestion produces fragments that are uniformly too large to clone.
(B) Generates overlapping fragments for chromosome walking and mapping.
(C) It prevents the restriction enzyme from digesting the vector DNA.
(D) It selectively degrades highly repetitive DNA sequences in the sample.

2016

**236.** What defines an expression library in contrast to a standard genomic library?
(A) cDNA inserted near a promoter for transcription/translation.
(B) The library uses only YAC vectors for large inserts.
(C) The clones are exclusively screened using radioactive DNA probes.
(D) The inserts are entirely non-coding regulatory regions of DNA.

2012

**237.** Which technique involves the transfer of DNA directly from bacterial colonies onto a nitrocellulose membrane for hybridization screening?
(A) Western blotting
(B) Plaque lifting
(C) Colony hybridization
(D) Southern blotting

NODIA

2007

**238.** In a plaque hybridization assay screening a bacteriophage library, what creates the clear zones (plaques) on the bacterial lawn?
(A) The toxic effect of the hybridization probe
(B) Lysis of the host bacterial cells by multiplying recombinant phages
(C) Antibiotic selection pressure
(D) The precipitation of X-gal

2001

**239.** Identify the INCORRECT statement regarding the application of a cDNA library.

into close proximity to enable energy transfer.
(B) A single probe is cleaved by the polymerase.
(C) The probe intercalates into the DNA, increasing its intrinsic fluorescence.
(D) The quencher is chemically dumped by the buffer.

2023
**185.** Consider the following statements about qPCR quantification.
I. A lower Ct value indicates a higher initial target concentration.
II. Absolute quantification requires a standard curve of known concentrations.
III. Relative quantification often normalizes target gene expression to a reference (housekeeping) gene.
IV. Amplification efficiency has no effect on the final Ct value.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, III and IV
(C) I, II and III
(D) II, III and IV

2007
**186.** Identify the INCORRECT statement regarding Molecular Beacons.
(A) They possess a stem-and-loop structure in the unhybridized state.
(B) They are consumed and degraded during the extension phase of qPCR.
(C) The loop portion contains the sequence complementary to the target.
(D) They offer high specificity, capable of distinguishing single nucleotide mismatches.

2010
**187.** What is the fundamental difference between standard PCR and quantitative Real-Time PCR (qPCR)?
(A) Standard measures plateau phase; qPCR measures exponential phase.
(B) Standard PCR uses RNA; qPCR uses DNA.
(C) Standard PCR requires thermal cycling; qPCR is isothermal.
(D) Standard PCR uses Taq polymerase; qPCR uses RNA polymerase.

2021
**188.** In microarray technology, what defines a "probe" based on standard nomenclature?
(A) The fluorescently labeled nucleic acid sample in solution
(B) The known DNA sequence immobilized onto the solid surface
(C) The enzyme used for signal detection
(D) The hybridization buffer

2011
**189.** Which method is commonly used for in situ fabrication of high-density oligonucleotide microarrays (e.g., Affymetrix GeneChips)?
(A) Robotic spotting of pre-synthesized PCR products
(B) Photolithography utilizing light-directed chemical synthesis
(C) Electro-spraying of RNA molecules
(D) Manual pipetting into distinct wells

2015
**190.** In a typical two-color cDNA microarray experiment, what do the green and red fluorescent dyes (e.g., Cy3 and Cy5) represent?
(A) Two distinct samples hybridized competitively to the array.
(B) Differentiation between live and dead cells in situ.
(C) Distinction between DNA and RNA molecules in the sample.
(D) Relative concentrations of probes versus target molecules.

2003
**191.** What is the primary purpose of data normalization (e.g., Lowess normalization) in microarray analysis?
(A) Removes technical variation and systematic biases from data.
(B) To increase the intensity of weak signals arbitrarily in the set.
(C) To align the sequences to a specific reference genome.
(D) To design better probes for future generations of arrays.

2025
**192.** Identify the INCORRECT statement about protein microarrays.
(A) They can be used to study protein-protein interactions and kinase activities.
(B) They are strictly limited to the detection of nucleic acid sequences.
(C) Capture molecules can include antibodies, aptamers, or recombinant proteins.
(D) Maintaining protein stability and native conformation on the chip surface is a major challenge.

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2001

**155.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).

**Assertion (A):** RFLP is a co-dominant marker system.

**Reason (R):** RFLP can distinguish heterozygotes from homozygotes based on the banding pattern on a blot.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2002

**156.** Random Amplified Polymorphic DNA (RAPD) differs from RFLP in that:

(A) RAPD requires prior knowledge of the target DNA sequence.
(B) PCR-based method using short arbitrary primers without sequence knowledge.
(C) RAPD utilizes radioactive probes for detection of targets.
(D) RAPD yields co-dominant markers exclusively in all populations.

2006

**157.** Which feature is considered a significant disadvantage of the RAPD marker system?

(A) High cost of arbitrary primers
(B) Requirement for large amounts of highly purified high-molecular-weight DNA
(C) Low reproducibility across different laboratories and PCR platforms
(D) Inability to detect any polymorphism

2023

**158.** Amplified Fragment Length Polymorphism (AFLP) combines elements of which two techniques?

(A) RFLP and RAPD
(B) RFLP and PCR
(C) Microarrays and PCR
(D) Sanger sequencing and Southern blotting

2019

**159.** In the AFLP methodology, what are the adapters ligated to?

(A) The ends of randomly sheared DNA fragments in the sample.
(B) Cohesive ends generated by restriction enzyme digestion.
(C) The RNA transcripts prior to reverse transcription steps.
(D) The PCR primers used for the initial amplification phase.

2021

**160.** Identify the INCORRECT statement comparing RFLP, RAPD, and AFLP.

(A) RFLP requires Southern blotting; RAPD and AFLP do not.
(B) AFLP generally produces a higher multiplex ratio (more bands per reaction) than RAPD.
(C) RAPD markers are typically dominant, meaning they cannot distinguish heterozygotes from dominant homozygotes.
(D) AFLP markers are exclusively co-dominant in all plant species.

2008

**161.** Which type of variation is directly responsible for generating an RFLP?

(A) Alternative splicing of mRNA
(B) Point mutations that create or destroy a restriction enzyme recognition site
(C) Variations in protein phosphorylation
(D) Different rates of transcription

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2008

**162.** Minisatellites, often used in classic DNA fingerprinting, are technically referred to as:

(A) Short Tandem Repeats (STRs)
(B) Variable Number of Tandem Repeats (VNTRs)
(C) Single Nucleotide Polymorphisms (SNPs)
(D) Long Interspersed Nuclear Elements (LINEs)

2009

**163.** How do microsatellites (SSRs) typically differ structurally from minisatellites (VNTRs)?

(A) SSR repeat units are shorter (typically 1-6 bp) compared to VNTR repeat units (10-100 bp).
(B) SSRs are located only in telomeres, while VNTRs are centromeric.
(C) SSRs consist entirely of RNA.
(D) SSRs do not exhibit length polymorphism.

2009

**164.** What is the primary molecular cause of high polymorphism levels in microsatellite (SSR) loci?

(A) Retroviral integration
(B) DNA polymerase slippage during replication
(C) Frequent double-strand breaks
(D) Non-homologous end joining

2024

**165.** Match the molecular marker in List I with its primary analytical principle in List II.

2017

**202.** Surface Plasmon Resonance (SPR) biosensors measure molecular interactions in real-time by detecting changes in which physical property?
(A) The emission of fluorescent light
(B) The generation of heat during a chemical reaction
(C) The refractive index near a sensor surface upon analyte binding
(D) The radioactive decay of an isotope

2014

**203.** Which parameter can be directly determined from a sensogram generated by an SPR experiment?
(A) The precise amino acid sequence of the binding partners
(B) The association (kon) and dissociation (koff) rate constants of the interaction
(C) The three-dimensional structure of the complex
(D) The absolute molecular weight of the analyte

2022

**204.** What acts as the bioreceptor in an electrochemical glucose biosensor?
(A) A platinum electrode
(B) Glucose oxidase
(C) Hydrogen peroxide
(D) A gold nanoparticle

2006

**205.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).
**Assertion (A):** Quantum dots are highly suitable for multiplexed cellular imaging.
**Reason (R):** Quantum dots possess narrow, size-tunable emission spectra and a broad excitation spectrum.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2025

**206.** Identify the INCORRECT feature of gold nanoparticles (AuNPs) in nanodiagnostics.
(A) They exhibit localized surface plasmon resonance (LSPR).
(B) They are highly toxic and degrade rapidly in aqueous solutions.
(C) They undergo a distinct color change (e.g., red to blue) upon aggregation.
(D) They can be easily functionalized with thiolated oligonucleotides.

2009

**207.** Which optical property gives quantum dots a significant advantage over traditional organic fluorophores for long-term imaging?
(A) They are highly resistant to photobleaching.
(B) Their emission spectrum shifts continuously over time.
(C) They exclusively emit in the ultraviolet range.
(D) They require two-photon excitation for any fluorescence.

2025

**208.** In a sandwich ELISA configuration adapted for a biosensor, what must the two antibodies recognize?
(A) The identical epitope on the analyte, competing for binding in situ.
(B) Distinct, non-overlapping epitopes on the target analyte.
(C) The transducer surface directly for signal generation.
(D) The secondary reporter enzyme exclusively for quantification.

2009

**209.** What defines the principle of Luminex bead-based multiplex assays?
(A) Microspheres dyed with unique fluorophore ratios for capture.
(B) Using identical microspheres differentiated only by physical size.
(C) Using a single flat microarray surface dotted with specific probes.
(D) Using magnetic beads that emit radio waves for detection.

2025

**210.** In a Luminex flow cytometry system, what are the functions of the two distinct lasers?
(A) One identifies the analyte bead; the other quantifies binding.
(B) One vaporizes the bead; the other detects the resulting mass.
(C) One measures forward scatter; the other side scatter exclusively.
(D) One controls fluidics; the other controls data logging steps.

2002

**211.** Which component in a lateral-flow immunochromatographic assay (e.g., a pregnancy test) allows for visual detection of the target?
(A) A radioactive isotope conjugated to the

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2008

**175.** Consider the sequence of events in RT-PCR (Reverse Transcription PCR).

I. RNase H degrades the RNA template.
II. Reverse transcriptase synthesizes a first-strand cDNA.
III. Taq polymerase amplifies the specific cDNA target.
IV. Oligo(dT) or random primers anneal to the RNA.

Which of the following is the correct order of arrangement?

(A) IV, II, I, III
(B) II, IV, I, III
(C) IV, I, II, III
(D) I, IV, II, III

2018

**176.** Which issue frequently arises if the extension time during PCR is significantly shorter than required for the amplicon size?

(A) Primer dimer formation dominates the reaction.
(B) Generation of incomplete, truncated PCR products.
(C) The DNA template degrades completely.
(D) The Taq polymerase denatures prematurely.

2014

**177.** Identify the INCORRECT statement regarding Taq DNA polymerase.

(A) It was originally isolated from the thermophilic bacterium Thermus aquaticus.
(B) It functions optimally around 72°C.
(C) It possesses a robust 3' to 5' proofreading exonuclease activity.
(D) It tends to add a single adenine (A) overhang to the 3' end of amplicons.

2018

**178.** What critical role does Magnesium ($MG_2^+$) play in the PCR reaction?

(A) It buffers the pH of the reaction mixture.
(B) It acts as a necessary cofactor for the catalytic activity of DNA polymerase.
(C) It prevents the degradation of the primers.
(D) It specifically denatures the template DNA.

2019

**179.** In qPCR, what does the SYBR Green dye specifically bind to?

(A) The minor groove of double-stranded DNA
(B) Single-stranded DNA primers
(C) The active site of Taq polymerase
(D) RNA-DNA hybrids exclusively

2002

**180.** Which structural feature of a TaqMan probe allows it to function in qPCR?

(A) It intercalates directly into the DNA helix in situ.
(B) Probe cleavage by Taq exonuclease releases the fluorophore.
(C) It forms a hairpin loop that opens only upon heating.
(D) It covalently binds to the amplicon, shifting its mass.

2008

**181.** What defines the Ct (Threshold Cycle) or Cq value in a Real-Time PCR assay?

(A) The final cycle number of the PCR program
(B) The cycle at which the fluorescence signal crosses a defined background threshold, marking the exponential phase
(C) The temperature at which the amplicon melts
(D) The cycle at which the reaction plateau is reached

2011

**182.** Given below are two statements: one is labelled as Assertion (A) and the other is labelled as Reason (R).

**Assertion (A):** SYBR Green qPCR requires a post-amplification melt curve analysis.

**Reason (R):** SYBR Green binds non-specifically to all double-stranded DNA, including primer dimers and offF-target amplicons.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

NODIA

2010

**183.** Which qPCR probe chemistry relies on physical separation of fluorophore and quencher via a conformational change upon target hybridization, without probe cleavage?

(A) TaqMan probes for detection.
(B) SYBR Green I dye.
(C) Conformational Molecular Beacons.
(D) Intercalating ethidium bromide.

2010

**184.** In Fluorescence Resonance Energy Transfer (FRET) based qPCR assays (e.g., LightCycler probes), how is the signal generated?

(A) Two adjacent probes hybridize to the target, bringing a donor and acceptor fluorophore

the resin and the simultaneous removal of side-chain protecting groups?
(A) Piperidine
(B) Liquid ammonia
(C) Sodium hydroxide
(D) Concentrated Trifluoroacetic acid (TFA) with scavengers

2004

**221.** Which challenge is frequently encountered during the solid-phase synthesis of long peptides (>50 amino acids)?
(A) Accumulated incomplete couplings reduce final full-length yield.
(B) The resin support dissolves completely in the utilized reaction solvent.
(C) The N-terminus becomes irreversibly blocked by atmospheric oxygen.
(D) The peptide spontaneously folds into functional enzymes on the resin.

2015

**222.** Which reagent acts as a scavenger during the final TFA cleavage step in SPPS to prevent reactive carbocations from modifying the peptide?
(A) Triethylamine
(B) Water or ethanedithiol (EDT)
(C) Dichloromethane (DCM)
(D) Piperidine

2011

**223.** What is the primary purpose of designing a synthetic peptide with D-amino acids for therapeutic applications?
(A) To enhance its resistance to proteolytic degradation in vivo
(B) To increase its solubility in water
(C) To make it highly fluorescent
(D) To ensure it binds exclusively to DNA

2009

**224.** Consider the synthesis of a dipeptide using Fmoc-SPPS.
I. Deprotecting the Fmoc group from the resin-bound amino acid
II. Coupling the second Fmoc-amino acid
III. Washing the resin
IV. Activating the second Fmoc-amino acid
Which sequence represents the correct logical order for one cycle of addition?
(A) I, III, IV, II
(B) I, IV, II, III
(C) IV, I, II, III
(D) I, II, III, IV

2022

**225.** Consider the following statements about peptide synthesis.
I. SPPS occurs in the C-to-N direction.
II. Ribosomal translation occurs in the N-to-C direction.
III. Fmoc is removed by mild base.
IV. Boc is removed by mild base.
V. Side chain protecting groups prevent branching.
Which of the above statement(s) is/are correct?
(A) I, II, III, V
(B) I, II, IV, V
(C) II, III, IV, V
(D) I, III, IV, V

2003

**226.** In the context of nucleic acid isolation, what role does phenol play in a phenol-chloroform extraction?
(A) It precipitates the DNA out of the aqueous phase.
(B) It denatures proteins, causing them to partition into the organic phase or at the interphase.
(C) It degrades RNA exclusively.
(D) It breaks down the cell membrane.

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2009

**227.** Why is isoamyl alcohol frequently added to the phenol-chloroform mixture?
(A) To prevent foaming and stabilize the phase boundary
(B) To increase the density of the organic phase
(C) To act as a chelating agent for magnesium ions
(D) To neutralize the pH of the aqueous phase

2002

**228.** Which chemical is routinely utilized to precipitate nucleic acids from an aqueous solution in the presence of monovalent cations?
(A) Chloroform
(B) Isopropanol or Ethanol
(C) Sodium Dodecyl Sulfate (SDS)
(D) Ethylenediaminetetraacetic acid (EDTA)

2019

**229.** Identify the INCORRECT statement concerning the use of chaotropic salts (e.g., guanidinium thiocyanate) in RNA isolation.
(A) They are powerful protein denaturants.
(B) They strongly inhibit intrinsic RNases.
(C) They promote the binding of RNA to silica membranes.
(D) They specifically degrade genomic DNA

capture antibody in situ.
(B) Colored nanoparticles conjugated to the detection antibody.
(C) A fluorescent intercalating dye specific for the target protein.
(D) An enzyme that produces gas to displace the indicator line.

2019
**212.** In a standard positive result on a lateral-flow assay, which lines must appear colored?
(A) Only the test line
(B) Only the control line
(C) Both the test line and the control line
(D) Neither line

2009
**213.** What is the primary purpose of the control line in a lateral flow assay?
(A) Confirms wicking through the strip and reagent activity.
(B) To capture the target analyte independently of the test line.
(C) To quantify the exact concentration of the target analyte.
(D) To neutralize cross-reacting antigens within the sample.

2011
**214.** Identify the INCORRECT statement regarding point-oF-care (POC) rapid technologies.
(A) They are designed to provide actionable results quickly near the patient.
(B) Lateral flow tests often trade absolute quantitative precision for speed and simplicity.
(C) Microfluidic paper-based analytical devices (μPADs) are an emerging POC format.
(D) They generally require highly trained personnel and extensive sample preparation.

2016
**215.** Match the solid-phase peptide synthesis (SPPS) component in List I with its function in List II.
List I: (SPPS Component)
P. Merrifield resin
Q. Fmoc group
R. DCC or HATU
S. Piperidine
List II: (Function)
1. Base used for deprotection
2. Coupling reagent to activate the carboxyl group
3. Insoluble polymer support
4. Temporary alpha-amino protecting group
(A) P-4, Q-3, R-1, S-2
(B) P-3, Q-1, R-4, S-2
(C) P-1, Q-4, R-2, S-3
(D) P-3, Q-4, R-2, S-1

2023
**216.** In Fmoc solid-phase peptide synthesis, the peptide chain is elongated in which direction?
(A) From the N-terminus to the C-terminus
(B) From the C-terminus to the N-terminus
(C) Simultaneously from both ends
(D) Starting from the middle and growing outwards

2017
**217.** Why is the Boc-strategy for SPPS generally considered more hazardous or demanding than the Fmoc-strategy?
(A) Requires corrosive TFA for deprotection and lethal HF for cleavage.
(B) Boc chemistry relies on high-temperature organic solvents for reaction.
(C) Boc groups are highly radioactive isotopes of common elements.
(D) Boc synthesis only occurs in aqueous environments under high pressure.

2022
**218.** What is the fundamental advantage of utilizing a solid resin support in peptide synthesis (Merrifield's concept)?
(A) Excess reagents are easily removed by filtration and washing.
(B) It increases the biological activity of the synthesized peptide.
(C) It eliminates the need for any protecting groups during synthesis.
(D) It forces the peptide into a specific alpha-helical conformation.

2015
**219.** Identify the INCORRECT statement regarding synthetic peptides.
(A) They can be used as antigens to generate highly specific antibodies.
(B) They serve as lead compounds for the development of peptide therapeutics.
(C) SPPS allows for the incorporation of unnatural amino acids (e.g., D-amino acids).
(D) They cannot mimic the post-translational modifications of native proteins.

2015
**220.** During Fmoc SPPS, what reagent is typically used for the final cleavage of the peptide from

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(A) It primarily represents the actively transcribed genes of a specific tissue.
(B) Contains introns and extensive genomic regulatory regions.
(C) It is heavily dependent on the reverse transcriptase enzyme.
(D) It simplifies the cloning of eukaryotic genes into prokaryotic systems.

2019

**240.** Which modification is frequently applied to the 5' ends of cDNA molecules to facilitate their insertion into a vector?
(A) Inclusion of oligonucleotide linkers with specific restriction sites.
(B) Digestion with exonuclease III to create blunt ends.
(C) Poly-adenylation by poly-A polymerase in the reaction.
(D) Covalent linkage to a fluorescent dye for tracking.

2013

**241.** During cDNA synthesis, what is the specific role of RNase H?
(A) Nicks hybrid RNA for 3'-OH second-strand DNA primers.
(B) To degrade adventitious RNA contaminants prior to synthesis.
(C) To add the poly-A tail to the mature mRNA molecule.
(D) To digest the completed cDNA molecule after synthesis.

2005

**242.** What is the primary purpose of Chromatin Immunoprecipitation (ChIP)?
(A) To determine the entire proteome of a cell
(B) To purify high molecular weight genomic DNA
(C) To map the specific in vivo binding sites of proteins (e.g., transcription factors) on DNA
(D) To sequence highly modified proteins

2019

**243.** In a ChIP assay, what chemical is typically used to reversibly crosslink proteins to DNA in living cells?
(A) Ethidium bromide
(B) Formaldehyde
(C) Sodium hydroxide
(D) Phenol

2014

**244.** Identify the INCORRECT step in a standard ChIP-seq workflow.
(A) Formaldehyde crosslinking
(B) Sonication or enzymatic digestion to shear chromatin into smaller fragments
(C) Complete target DNA digestion by exonucleases before sequencing.
(D) Immunoprecipitation utilizing an antibody specific to the target protein

2023

**245.** Which technique evaluates global gene expression by directly counting the number of sequence tags generated from the 3' ends of transcripts?
(A) Serial Analysis of Gene Expression (SAGE)
(B) Microarray hybridization
(C) Chromosome Conformation Capture (3C)
(D) Northern Blotting

2008

**246.** How does RNA-seq fundamentally differ from DNA microarrays for transcriptome profiling?
(A) RNA-seq relies on continuous hybridization to predefined probes.
(B) Detects novel transcripts and splice variants without prior knowledge.
(C) RNA-seq cannot quantify expression levels accurately in situ.
(D) RNA-seq exclusively utilizes Sanger sequencing chemistry for reads.

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2017

**247.** What is the fundamental basis of a "gel shift" or Electrophoretic Mobility Shift Assay (EMSA)?
(A) A DNA-protein complex migrates more slowly through a non-denaturing gel than free DNA.
(B) Proteins are separated purely by isoelectric point.
(C) DNA is denatured to observe single-strand conformations.
(D) RNA relies on capillary action to migrate.

2012

**248.** In an EMSA, what confirms the specific identity of the protein bound to the DNA probe?
(A) A "supershift" generated by adding a specific antibody directed against the target protein
(B) The addition of ethidium bromide
(C) Boiling the sample before loading
(D) The complete disappearance of the DNA band

2011

**249.** Which method is most appropriate for precisely identifying the specific nucleotides within a DNA sequence where a transcription factor binds?
(A) Southern Blotting

(D) CRISPR functions independently of host cell repair machinery.

2010

**270.** In the context of RNA interference (RNAi), what is the function of the Dicer enzyme?
(A) It degrades single-stranded mRNA targets.
(B) It cleaves long double-stranded RNA (dsRNA) or short hairpin RNA (shRNA) into ~21-23 nucleotide siRNAs.
(C) It synthesizes the complementary RNA strand.
(D) It transports the RNA out of the nucleus.

2007

**271.** Which multi-protein complex incorporates the guide strand of an siRNA and facilitates the cleavage of the complementary target mRNA?
(A) The Spliceosome
(B) The Proteasome
(C) The RNA-Induced Silencing Complex (RISC)
(D) The Ribosome

2006

**272.** Identify the INCORRECT statement comparing siRNAs and miRNAs.
(A) siRNAs typically require perfect complementarity to their target mRNA and induce direct cleavage.
(B) miRNAs often have imperfect complementarity and primarily induce translational repression or mRNA deadenylation.
(C) miRNAs are synthetic and never naturally encoded in the host.
(D) Both are processed by the Dicer enzyme in the cytoplasm.

2009

**273.** Which feature distinguishes shRNAs (short hairpin RNAs) from synthetic siRNAs when used for gene silencing experiments?
(A) Vector expression allows for stable, long-term gene knockdown.
(B) shRNAs do not require processing by Dicer for function.
(C) shRNAs function exclusively in the nucleus of the cell.
(D) shRNAs act by inhibiting DNA polymerase enzyme activity.

2002

**274.** What constitutes the major therapeutic hurdle for widespread clinical application of RNAi?
(A) Extreme difficulty in designing specific siRNA sequences.
(B) Targeted delivery of unstable, charged RNA to specific tissues.
(C) The rapid mutation of Dicer enzymes in human populations.
(D) The irreversible alteration of the patient's genomic DNA.

2008

**275.** In flow cytometry, what does Forward Scatter (FSC) primarily measure?
(A) Approximate cell size or volume
(B) Cell granularity or internal complexity
(C) The intensity of specific surface marker expression
(D) DNA content

2018

**276.** What does Side Scatter (SSC) in flow cytometry primarily indicate?
(A) Cell volume
(B) Cell membrane potential
(C) The amount of fluorescent dye bound
(D) The internal complexity or granularity of the cell

2024

**277.** Which function defines Fluorescence-Activated Cell Sorting (FACS)?
(A) Isolating live cell populations based on fluorescent parameters.
(B) Purely analyzing the scatter properties of cells in a stream.
(C) Lysing cells and separating their proteins in an electric field.
(D) Sequencing the DNA of cells passing through a laser in situ.

2017

**278.** When analyzing the cell cycle via flow cytometry, which intercalating dye is most commonly utilized to quantify DNA content?
(A) FITC
(B) Cy5
(C) Coomassie Blue
(D) Propidium Iodide (PI)

2004

**279.** In a DNA histogram generated by flow cytometry, the large peak representing cells with a 2N DNA content corresponds to which cell cycle phase?
(A) $G_0/G_1$ phase
(B) S phase
(C) $G_2$/M phase
(D) Apoptosis

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(A) The donor and acceptor must be separated by at least 100 nanometers.
(B) Both fluorophores must be identical within the cell environment.
(C) Donor emission spectrum must significantly overlap acceptor excitation.
(D) The acceptor must be a radioactive isotope within the complex.

2005

**260.** What physical constraint defines the working distance of FRET (the Förster distance)?
(A) It is typically effective only over distances of 1 to 10 nanometers.
(B) It operates efficiently up to 1 millimeter.
(C) It requires the proteins to be covalently crosslinked.
(D) It relies on random diffusion in the cytoplasm.

2008

**261.** Phage display technology fundamentally links which two biological elements?
(A) Viral replication and bacterial host lysis
(B) Transcription and translation
(C) DNA synthesis and protein degradation
(D) Phage DNA genotype with displayed surface protein phenotype.

2004

**262.** In a phage display library, the gene of interest is typically fused to the gene encoding which structural component?
(A) The phage RNA polymerase
(B) A coat protein (e.g., pIII or pVIII of filamentous phage $M_{13}$)
(C) The host cell's ribosome
(D) The origin of replication

2019

**263.** What is the term for the iterative process of binding, washing, elution, and amplification used to isolate specific binding phages from a library?
(A) Southern blotting
(B) Panning
(C) Electroporation
(D) Flow cytometry sorting

2008

**264.** Identify the INCORRECT statement concerning CRISPR-Cas9 genome editing.
(A) Cas9 is an RNA-guided DNA endonuclease.
(B) sgRNA contains 20 nucleotides complementary to target DNA.
(C) Cas9 induces a targeted double-strand break in DNA.
(D) Cleavage requires a PAM sequence located upstream of guide binding.

2018

**265.** What dictates the precise location where Cas9 will cleave the target DNA?
(A) It cleaves randomly within any GC-rich region.
(B) It cleaves exactly 3 base pairs upstream of the PAM sequence.
(C) It cleaves at the first start codon it encounters.
(D) It cleaves exactly at the 5' end of the sgRNA.

2015

**266.** Following a Cas9-induced double-strand break, which cellular repair pathway is primarily utilized to introduce random insertions or deletions (indels) for gene knockout?
(A) Non-Homologous End Joining (NHEJ)
(B) Homology-Directed Repair (HDR)
(C) Base Excision Repair (BER)
(D) Mismatch Repair (MMR)

2023

**267.** If a researcher wishes to introduce a specific point mutation using CRISPR-Cas9, what must be provided in addition to the Cas9 protein and sgRNA?
(A) A donor DNA template containing the desired mutation flanked by homologous sequences
(B) A highly active exonuclease
(C) A retroviral integrase
(D) A high concentration of DNA ligase

2010

**268.** Which variant of Cas9 is used for CRISPR interference (CRISPRi) to repress transcription without cleaving the DNA?
(A) Nickase Cas9 (nCas9)
(B) Split Cas9
(C) Wild-type Cas9 lacking a PAM-interacting domain
(D) Catalytically dead Cas9 (dCas9) fused to a transcriptional repressor

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2007

**269.** What is a principal advantage of the CRISPR-Cas system over earlier targeted nucleases like ZFNs and TALENs?
(A) CRISPR does not require DNA binding for function.
(B) RNA-guided base pairing avoids complex protein engineering.
(C) CRISPR exclusively causes single-strand nicks in targets.

# ANSWERS

**1.** (C) 50 μg/mL
Ultraviolet spectrophotometry quantifies nucleic acids by measuring absorbance at 260 nm. For double-stranded DNA, an optical density of one corresponds to a concentration of 50 micrograms per milliliter when measured in a standard one-centimeter path length cuvette. This value is a standard conversion factor used in molecular biology laboratories for concentration calculations and downstream genetic applications.

**2.** (C) Proteins absorb maximally at 280 nm, decreasing the overall ratio.
Nucleic acid purity is assessed using the ratio of absorbance at 260 nm and 280 nm. Pure DNA typically shows a ratio between 1.8 and 2.0. Proteins absorb light maximally at 280 nm due to aromatic amino acids like tyrosine and tryptophan. Consequently, significant protein presence increases 280 nm absorbance, lowering the ratio towards 1.5.

**3.** (A) Prevents contamination overestimation via target-specific dyes.
Fluorometric quantification methods utilize dyes that emit fluorescence only when bound to specific target molecules like DNA or RNA. Unlike ultraviolet spectrophotometry, which measures total absorbance including contaminants like proteins or free nucleotides, fluorometry provides superior accuracy by minimizing overestimation. This specificity ensures precise measurement of the desired nucleic acid species in complex biological samples.

**4.** (D) Exhibits high sensitivity to interference by common detergent reagents.
The bicinchoninic acid assay is a colorimetric method used to quantify total protein concentration. While it relies on the reduction of copper ions in an alkaline environment, it is notably compatible with many common detergents and denaturants. Stating that it exhibits high sensitivity to interference by these reagents is inaccurate, as it is often preferred over other assays.

**5.** (A) Both A and R are true and R is the correct explanation of A.
The Bradford assay utilizes Coomassie Brilliant Blue G-250 dye, which shifts its absorbance maximum from 465 nm to 595 nm upon binding to proteins. At high protein concentrations, the depletion of the free dye pool limits additional binding events, resulting in a non-linear standard curve. The relationship between absorbance and concentration thus becomes inconsistent at these elevated levels.

**6.** (C) Folin-Ciocalteu reagent
The Lowry protein assay is a biochemical procedure used to determine total protein levels in a solution. This method involves a two-step reaction where copper ions react with peptide bonds, followed by the reduction of the Folin-Ciocalteu reagent. This reduction process produces a blue color, which is then measured spectrophotometrically to quantify the amount of protein present.

**7.** (D) Phenol or chaotropic salts
The absorbance ratio at 260 nm and 230 nm serves as an indicator of nucleic acid purity regarding organic contaminants. A low ratio, such as 1.2, typically signals the presence of residual phenol, EDTA, or chaotropic salts used during extraction. Pure RNA samples should ideally exhibit a ratio greater than 2.0 for optimal performance in downstream molecular experiments.

**8.** (A) Arginine and aromatic residues
The Bradford assay relies on the binding of Coomassie Brilliant Blue G-250 dye to specific protein residues. The dye interacts primarily with basic amino acids, particularly arginine, through electrostatic attractions. Additionally, it binds to aromatic amino acids like phenylalanine, tryptophan, and tyrosine via van der Waals forces. These interactions cause the characteristic color change from reddish-brown to blue.

**9.** (B) Utilizing the Bradford assay over the Lowry assay.
Protein quantification assays vary in their tolerance to chemical additives. The Bradford

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2025

**280.** Identify the INCORRECT statement regarding compensation in flow cytometry.
(A) It is required when utilizing multiple fluorophores simultaneously.
(B) It eliminates the need for proper experimental controls.
(C) It mathematically corrects for the spectral overlap (spillover) of one fluorophore's emission into another fluorophore's detector.
(D) Proper compensation requires single-stained control samples.

2001

**281.** What defines an "aptamer"?
(A) A short, highly structured single-stranded DNA or RNA molecule capable of binding specifically to a target molecule with high affinity
(B) A specific class of monoclonal antibodies
(C) A lipid nanoparticle used for drug delivery
(D) A highly repetitive region of genomic DNA

2011

**282.** By which in vitro evolutionary process are aptamers typically isolated from large random sequence libraries?
(A) SELEX (Systematic Evolution of Ligands by Exponential Enrichment)
(B) Phage display
(C) Edman degradation
(D) Golden Gate Assembly

2002

**283.** Which feature provides aptamers with a distinct advantage over traditional antibodies in diagnostic biosensors?
(A) Aptamers are small, synthetic, and easily modified with tags.
(B) Aptamers can only be produced in vivo using animal hosts.
(C) Aptamers are exclusively composed of amino acids in a chain.
(D) Aptamers permanently and covalently bind their target molecules.

2001

**284.** In SELEX, what occurs immediately after the incubation of the random nucleic acid library with the target molecule?
(A) The target is sequenced.
(B) The entire mixture is injected into an animal.
(C) The bound sequences are translated into proteins.
(D) The unbound nucleic acids are washed away, and the bound sequences are eluted.

2024

**285.** Which statement accurately describes a SOMAmer (Slow OfF-rate Modified Aptamer)?
(A) Contains modified nucleotides to enhance protein binding.
(B) An aptamer exclusively utilized to bind RNA target molecules.
(C) A hybrid molecule composed of halF-antibody and halF-nucleic acid.
(D) An aptamer lacking secondary structure in its active state.

2021

**286.** What is the fundamental basis of a riboswitch?
(A) Regulatory mRNA segment that binds small molecules for control.
(B) A small synthetic RNA used to switch off DNA replication in situ.
(C) An enzyme that converts RNA back into DNA in the cell.
(D) A membrane receptor that senses ribose sugars in the media.

2021

**287.** Identify the INCORRECT characteristic of modern lab-on-a-chip (microfluidic) devices.
(A) They process extremely small volumes of fluids (picoliters to microliters).
(B) They integrated multiple laboratory functions on a single chip.
(C) They allow for high-throughput parallel processing.
(D) They suffer from extremely slow reaction times compared to macroscopic assays.

2022

**288.** In soft lithography for fabricating microfluidic devices, which transparent, flexible elastomer is most commonly used?
(A) Polydimethylsiloxane (PDMS)
(B) Polyacrylamide
(C) Agarose
(D) Nitrocellulose

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2008

**289.** Which driving force is often utilized in microfluidic devices to precisely manipulate fluid flow without mechanical pumps?
(A) Gravity alone
(B) Electro-osmotic flow generated by an applied electric field
(C) Magnetic levitation
(D) Centrifugal force exclusively

(B) Standard PCR
(C) Flow Cytometry
(D) DNase I Footprinting

2012
**250.** Identify the INCORRECT statement regarding DNase I Footprinting.
(A) One strand of the target DNA is end-labeled, typically with a radioisotope or fluorophore.
(B) Exposes DNA to excessive DNase I to degrade all regions.
(C) The binding of the specific protein protects the underlying DNA from cleavage.
(D) The resulting fragments are analyzed on a high-resolution denaturing polyacrylamide gel.

2015
**251.** What does the "footprint" represent on the final autoradiograph in a footprinting assay?
(A) A blank area (missing bands) in the cleavage ladder corresponding to the protein-bound protected sequence
(B) A darkened region with extra bands representing enhanced cleavage
(C) The position of the unbound protein in the gel
(D) The un-cleaved intact DNA at the very top of the gel

2006
**252.** Which reporter gene assay utilizes a substrate that emits light without the need for an external excitation source?
(A) Green Fluorescent Protein (GFP)
(B) Beta-galactosidase
(C) Chloramphenicol acetyltransferase (CAT)
(D) Luciferase

2017
**253.** In a dual-luciferase reporter assay, what is the typical role of the Renilla luciferase?
(A) It acts as an internal control for normalization of transfection efficiency and cell viability.
(B) It serves as the primary indicator of the experimental promoter's activity.
(C) It selectively inhibits the Firefly luciferase.
(D) It provides the substrate (luciferin) required for the assay.

2005
**254.** What is the primary biochemical reaction catalyzed by Firefly luciferase?
(A) Hydrolysis of lactose into glucose and galactose molecules.
(B) ATP-dependent oxidation of luciferin yielding photons.
(C) Transfer of an acetyl group to chloramphenicol antibiotic.
(D) Reduction of a tetrazolium salt to colored formazan.

2015
**255.** Identify the INCORRECT statement regarding the Yeast Two-Hybrid (Y2H) system.
(A) It is an in vivo technique designed to detect direct physical interactions between two proteins.
(B) It utilizes a split transcription factor (e.g., GAL4), separating the DNA-binding domain from the activation domain.
(C) Interaction of the "bait" and "prey" proteins reconstitutes a functional transcription factor, driving reporter gene expression.
(D) It requires the two interacting proteins to be localized to the cell membrane.

2004
**256.** In the Yeast Two-Hybrid system, what term describes the target protein fused to the DNA-Binding Domain (DBD)?
(A) The Bait
(B) The Prey
(C) The Reporter
(D) The Activator

2007
**257.** Which phenomenon can cause false positives in a standard Yeast Two-Hybrid assay?
(A) The "prey" protein degrades immediately in the yeast cell.
(B) The yeast cells lack the GAL4 promoter sequence entirely.
(C) Proteins require specific mammalian post-translation modifications.
(D) Bait protein has intrinsic activity and activates reporters alone.

2001
**258.** Which advanced technique allows for the detection of protein-protein interactions in living mammalian cells by monitoring the non-radiative transfer of energy between two fluorophores?
(A) Co-immunoprecipitation
(B) Surface Plasmon Resonance (SPR)
(C) Yeast Two-Hybrid
(D) Fluorescence Resonance Energy Transfer (FRET)

2025
**259.** For efficient FRET to occur, which condition must strictly be met?

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probes in the pore.

2014

**299.** What distinguishes the motor protein used in Oxford Nanopore technology from the pore itself?

(A) It generates the electrical current.
(B) Unwinds dsDNA and controls translocation speed through the pore.
(C) It binds specifically to methylated cytosines.
(D) It strictly prevents RNA from entering the pore.

2017

**300.** Which bioinformatic process is uniquely critical for assembling the highly accurate but error-prone raw reads produced by single-molecule long-read sequencing technologies?

(A) Trimming solely based on base quality scores from terminators.
(B) Assembly algorithms using long overlaps to average random errors.
(C) Ignoring all reads over 1000 base pairs in the data set.
(D) Converting all reads into simulated short Illumina reads.

**********

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2022
**290.** What is a primary application of droplet-based microfluidics (e.g., in single-cell RNA sequencing like Drop-seq)?
(A) To freeze cells instantly for microscopy study.
(B) Encapsulating cells in partitioned oil droplets for reactions.
(C) To measure the macroscopic viscosity of blood samples.
(D) To isolate large intact tissue samples from the host.

2014
**291.** Which limitation is generally associated with using PDMS in microfluidic applications involving small hydrophobic drug molecules?
(A) It is completely opaque to visible light wavelengths.
(B) It shatters easily under slight mechanical pressure.
(C) It conducts electricity too readily for most assays.
(D) PDMS absorbs small hydrophobic molecules, altering concentrations.

2017
**292.** In a microfluidic continuous-flow PCR device, how is thermal cycling achieved?
(A) Sample pumping through fixed temperature zones on the chip.
(B) The entire device is sequentially heated and cooled in cycles.
(C) A laser continuously pulses the static fluid in the chamber.
(D) The fluid relies on internal exothermic chemical reactions.

2005
**293.** Which application demonstrates the utility of "organ-on-a-chip" technology?
(A) Storing whole organs prior to surgical transplantation steps.
(B) Replicating functional organ units in vitro for physiology modeling.
(C) Sequencing the entire genome of an organ rapidly in situ.
(D) Replacing failed organs directly in a human patient.

2004
**294.** Identify the INCORRECT statement regarding digital PCR (dPCR).
(A) It partitions the sample into thousands to millions of separate, tiny micro-reactions prior to amplification.
(B) It relies on Poisson statistics to calculate the absolute number of target DNA molecules present.
(C) It provides higher precision and sensitivity for detecting rare alleles compared to standard bulk qPCR.
(D) It requires a standard curve for absolute quantification.

2010
**295.** What is the fundamental requirement for a successful digital PCR assay regarding sample partitioning?
(A) Each partition must contain exactly one target molecule in it.
(B) Dilution such that a fraction of partitions receives zero targets.
(C) The partitions must all be of different volumes in the assay.
(D) The DNA must be completely single-stranded before partitioning.

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2012
**296.** In droplet digital PCR (ddPCR), how are the positive partitions distinguished from the negative partitions after end-point amplification?
(A) By mass spectrometry
(B) By sequencing the contents of each droplet
(C) By droplet flow through fluorescence detectors for amplitude.
(D) By visual inspection under a standard light microscope.

2025
**297.** Which technology utilizes zero-mode waveguides (ZMWs) specifically designed to observe individual DNA polymerase molecules incorporating fluorescently labeled nucleotides in real time?
(A) Illumina MiSeq
(B) Oxford Nanopore MinION
(C) Pacific Biosciences (PacBio) RS/Sequel
(D) Affymetrix GeneChip

2005
**298.** In nanopore sequencing, what causes the characteristic disruption in the steady ionic current across the membrane?
(A) Physical blockage of the pore by nucleobases during translocation.
(B) The release of pyrophosphate and subsequent generation of light.
(C) The cleavage of the DNA backbone by the pore structure.
(D) The binding of complementary fluorescent

assay is generally more robust in the presence of reducing agents compared to the Lowry or BCA methods, which rely on copper reduction chemistry. Using the Bradford method effectively avoids interference from reagents like beta-mercaptoethanol or dithiothreitol, ensuring more accurate protein concentration measurements in such samples.

**10.** (B) The $A_2$60/280 ratio is 2.0, representing a high purity level.
Calculating the ratio of absorbance at 260 nm to 280 nm involves dividing the first value by the second. For these measurements, the resulting ratio is 2.0, which indicates a very high level of purity in the DNA sample. A ratio in this range suggests that the preparation is free from significant protein or phenol contamination during isolation.

**11.** (A) Bradford assay
The Bradford assay is distinct from other protein quantification methods because it involves the direct, non-covalent binding of a dye to protein molecules. In contrast, the Biuret, Lowry, and BCA assays rely on the chemical reduction of copper ions catalyzed by peptide bonds. The Bradford method represents a rapid, end-point binding equilibrium rather than a time-dependent catalytic process.

**12.** (C) Requires standard curves for each specific dye batch and run.
Fluorometric methods offer high sensitivity and specificity for nucleic acids but require careful calibration. Because the fluorescence signal depends on specific dye-target interactions and environmental factors, researchers must generate a standard curve for each batch of reagents and every individual run. This ensures that the measured intensity is accurately converted to concentration, maintaining consistency across different experimental setups.

**13.** (B) 10% Polyacrylamide
Polyacrylamide gels are the preferred matrix for resolving small DNA fragments ranging from 50 to 500 base pairs. Due to the small, uniform pore sizes formed by the cross-linking of acrylamide and bis-acrylamide, this medium provides much higher resolution than agarose. This allows for the clear separation of molecules that differ by only a few nucleotides in length.

**14.** (C) Imparts a uniform negative charge-to-mass ratio to proteins.
Sodium Dodecyl Sulfate is an anionic detergent used to denature proteins and coat them with a uniform negative charge. This process effectively masks the intrinsic charge of the amino acids and ensures that the charge-to-mass ratio is consistent across all proteins. Consequently, migration through the gel matrix becomes solely dependent on the molecular weight of the polypeptide chains.

**15.** (B) P-3, Q-1, R-4, S-2
Electrophoresis methods resolve molecules based on distinct physical properties. Denaturing techniques like SDS-PAGE separate proteins by molecular mass alone, whereas native PAGE depends on both charge and size. Isoelectric focusing utilizes a pH gradient to separate proteins at their isoelectric point. Capillary electrophoresis is driven by electro-osmotic flow and electrophoretic mobility, allowing for rapid and highly sensitive molecular analysis.

**16.** (D) Molecular weight
Two-dimensional polyacrylamide gel electrophoresis involves two sequential separation steps. The first dimension utilizes isoelectric focusing to separate proteins based on their charge or isoelectric point. The second dimension, typically performed using SDS-PAGE, separates these proteins according to their molecular weight. This combination allows for the high-resolution mapping of complex protein mixtures within a single gel system.

**17.** (A) Tris-Acetate-EDTA (TAE)
Tris-Acetate-EDTA is a common buffer system utilized during agarose gel electrophoresis for nucleic acids. The Tris component provides a stable pH, while acetate acts as an electrolyte to facilitate conductivity for the electric current. The addition of EDTA chelates divalent metal ions, which serves to inhibit DNA-degrading enzymes, ensuring the integrity of the samples during the migration process.

**18.** (C) Resolves single-base differences via uniform, consistent pores.
Polyacrylamide is chosen for separating small nucleic acid molecules because of its superior resolution capabilities. The chemical cross-linking of acrylamide monomers creates a matrix with highly uniform and consistent pores. These

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small pores allow for the precise separation of DNA or RNA fragments that differ by as little as a single base, which is not achievable using agarose.

**19.** (A) Separation is driven purely by size exclusion.
Capillary electrophoresis is a versatile technique that separates analytes based on their electrophoretic mobility and the effects of electro-osmotic flow. Stating that separation is driven purely by size exclusion is incorrect, as the charge-to-mass ratio and surface interactions also play critical roles. The narrow fused-silica capillaries allow for high electric fields and efficient heat dissipation during the analytical process.

**20.** (D) Ammonium persulfate (APS)
The polymerization of acrylamide and bis-acrylamide to form a gel matrix requires a free radical source. Ammonium persulfate acts as the initiator by generating sulfate radicals upon dissolution. These radicals then react with the acrylamide monomers to start the chain reaction. TEMED is typically added to catalyze this process, ensuring that the gel solidifies efficiently for subsequent molecular separation.

**21.** (B) The protein will migrate as a single larger intact complex.
SDS-PAGE typically requires a reducing agent like beta-mercaptoethanol to break disulfide bonds that hold multimeric protein complexes together. If this reagent is omitted, the individual subunits remain covalently linked. As a result, the protein will migrate through the gel as a single, larger intact complex rather than separating into its component polypeptide chains according to their individual molecular weights.

**22.** (C) Total acrylamide concentration and the ratio of bis-acrylamide.
The physical characteristics of a polyacrylamide gel are determined by its chemical composition. Specifically, the total concentration of acrylamide and the ratio of the cross-linker, bis-acrylamide, dictate the resulting pore size. Higher concentrations of these monomers create a denser matrix with smaller pores, which is ideal for resolving small molecules, while lower concentrations allow for larger molecules to pass.

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**23.** (D) Denatures genomic DNA and cellular protein content.
During the process of plasmid isolation, Solution II containing sodium hydroxide and sodium dodecyl sulfate is added to the cell suspension. This alkaline mixture serves to denature the genomic DNA and cellular proteins by disrupting hydrogen bonds and protein structures. While the large chromosomal DNA becomes linearized and denatured, the smaller circular plasmid DNA remains topologically linked for later recovery.

**24.** (A) Sharing the same replication or partitioning mechanisms
Plasmid incompatibility occurs when two different plasmids cannot be stably maintained within the same bacterial cell line. This phenomenon typically happens because the plasmids share identical replication or partitioning mechanisms. Competition for the same host factors or regulatory elements leads to the eventual loss of one plasmid over successive generations, ensuring that only one type remains dominant in the cell.

**25.** (D) I, II, and III
In a cesium chloride-ethidium bromide gradient, linear and supercoiled DNA exhibit different buoyant densities. Ethidium bromide intercalates more effectively into linear DNA, reducing its density more than it does for supercoiled plasmids, which have constrained unwinding. Consequently, supercoiled DNA reaches a higher buoyant density. Furthermore, RNA molecules are much denser and will pellet at the bottom of the tube.

**26.** (B) Antisense RNA-mediated control
The $ColE_1$ plasmid regulates its copy number through a sophisticated mechanism involving antisense RNA molecules. Replication is initiated by an RNA primer, but this process is controlled by the interaction between two RNA transcripts, RNA I and RNA II. By binding to the primer, the antisense RNA prevents the initiation of DNA synthesis, thereby maintaining a consistent number of plasmid copies.

**27.** (B) Relaxed plasmids require continuous protein synthesis for their replication.
Plasmids are categorized by their replication control mechanisms. Stringent plasmids are kept at low copy numbers, while relaxed plasmids can reach high levels. It is incorrect to claim that relaxed plasmids require continuous protein

synthesis for replication; in fact, they often continue to replicate even when host protein synthesis is inhibited. Episomes are a specific type capable of genomic integration.

**28.** (A) Neutralizes pH and precipitates chromosomal DNA and proteins.
Solution III, which typically contains potassium acetate, is used during the neutralization step of plasmid isolation. The addition of this reagent lowers the pH, allowing the smaller plasmid DNA to renature correctly. Simultaneously, the large chromosomal DNA and cellular proteins precipitate out of the solution as an insoluble complex, which can then be easily removed by centrifugation from the lysate.

**29.** (C) Par operon proteins
Low-copy-number plasmids require active mechanisms to ensure that at least one copy is inherited by each daughter cell during division. This process is managed by proteins encoded by the partitioning (Par) operon. These proteins act like a primitive mitotic spindle, physically separating the plasmid copies and moving them to opposite poles of the bacterial cell before the septum forms.

**30.** (D) Relies less on host replication factors such as the protein DnaA.
High-copy-number plasmids are characterized by their ability to replicate extensively within a host cell. Unlike low-copy-number plasmids, which often require specific host replication factors like the DnaA protein and strict partitioning systems, high-copy variants rely less on these host-regulated elements. This relative independence allows them to reach much higher concentrations in the bacterial cytoplasm without being limited by cellular machinery.

**31.** (C) Shared origin of replication and repressor control mechanism.
Two plasmids are considered incompatible if they cannot coexist in a single bacterial cell without selective pressure. This instability usually arises when both plasmids utilize the same origin of replication and identical repressor control mechanisms. As the host cell divides, the plasmids compete for the same regulatory machinery, leading to the random segregation and eventual loss of one plasmid type.

**32.** (B) The lack of free ends to relieve topological tension
Covalently closed circular DNA possesses a unique topological constraint because it lacks free ends. During ethidium bromide intercalation, the double helix must unwind to accommodate the dye molecules. In circular plasmids, this unwinding is limited by the accumulation of topological tension, which prevents the uptake of as much dye as linear DNA. This difference allows for separation based on buoyant density.

**33.** (D) RNase A
During the initial stages of plasmid isolation, it is common to include RNase A in the resuspension buffer, also known as Solution I. This enzyme is essential for degrading cellular RNA molecules that are released during lysis. By removing RNA early in the process, the final plasmid DNA product is significantly purer and free from contaminating ribonucleic acid fragments.

**34.** (A) An origin of replication and its associated control elements.
In plasmid biology, a replicon is defined as the minimal genetic unit capable of autonomous replication within a host cell. It consists of a specific origin of replication sequence along with all the associated cis-acting and trans-acting control elements required to initiate DNA synthesis. The replicon determines the plasmid's host range, copy number, and compatibility with other genetic elements.

**35.** (C) I, II and IV
Restriction enzymes are classified into distinct types based on their subunit structure and cleavage sites. Type I enzymes require ATP and S-adenosylmethionine to cut DNA at random locations far from their recognition sequence. Type II enzymes are most common in cloning, cutting at specific sites. Type IIS enzymes are unique because they cleave at a defined distance outside their recognition sequence.

**36.** (A) Type II
Type II restriction endonucleases are the primary tools used in recombinant DNA technology and molecular cloning. Their widespread utility stems from their ability to recognize specific palindromic DNA sequences and cleave the DNA at precise, predictable positions within or near those sites. This accuracy allows researchers to

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DNA molecules that are approximately 37 to 52 kilobases in length. This constraint restricts the amount of foreign DNA that can be successfully encapsulated and transferred.

**55.** (A) P-1, Q-2, R-3, S-4
Different cloning vectors are designed to accommodate specific sizes of DNA inserts. Standard plasmids typically handle fragments up to 15-20 kb, while cosmids can store approximately 35-45 kb. For much larger genomic projects, Bacterial Artificial Chromosomes are used for inserts up to 300 kb, and Yeast Artificial Chromosomes are employed for extremely large fragments exceeding 1000 kb in length.

**56.** (D) Two distinct origins of replication for different host organisms.
A shuttle vector is a versatile genetic tool designed to replicate in two different host organisms, such as bacteria and yeast. This ability is made possible by the inclusion of two distinct origins of replication, each specific to one of the host species. This allows researchers to manipulate DNA in one organism and then move it to another for functional study.

**57.** (A) They contain both plasmid and filamentous phage origins of replication.
Phagemids are hybrid vectors that contain genetic elements from both plasmids and filamentous phages. They possess a standard plasmid origin of replication for double-stranded maintenance in bacteria and a phage origin for the production of single-stranded DNA. When a host cell is co-infected with a helper phage, the phagemid DNA can be packaged into phage particles as single-stranded molecules.

**58.** (B) Binds the lac repressor to allow lacZ-alpha peptide expression.
In the blue-white screening method, IPTG serves as a non-metabolizable inducer of the lac operon. It functions by binding to the lac repressor protein, which causes the repressor to release from the operator sequence. This allows the host cell's machinery to transcribe the lacZ-alpha peptide encoded on the plasmid, which is essential for forming the functional beta-galactosidase enzyme during screening.

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**59.** (B) The foreign DNA is inserted into a selectable marker gene, disrupting its function.
Insertional inactivation is a screening technique used to identify recombinant clones. It involves inserting foreign DNA into the middle of a specific selectable marker gene, such as an antibiotic resistance gene or the lacZ gene. This insertion disrupts the gene's coding sequence, causing it to lose its function. The resulting change in the host's phenotype allows researchers to distinguish recombinants.

**60.** (A) Plasmid alpha-peptide and host omega-peptide form beta-gal.
Alpha-complementation is a biochemical phenomenon used to identify recombinant bacteria. It occurs when a small, plasmid-encoded alpha-peptide interacts with a truncated, host-encoded omega-peptide. Together, these two inactive fragments assemble to form a fully functional beta-galactosidase enzyme. This enzyme can then cleave substrates like X-gal to produce a blue color, indicating that the plasmid is present and the alpha-peptide is intact.

**61.** (C) Disrupted lacZ-alpha prevents X-gal cleavage by beta-gal.
In blue-white screening, successful ligation of an insert into the multiple cloning site disrupts the lacZ-alpha gene. As a result, the functional alpha-peptide is not produced, and the beta-galactosidase enzyme cannot be reconstituted via alpha-complementation. Without the active enzyme, the chromogenic substrate X-gal remains uncleaved, leading to the formation of white colonies, which identifies them as recombinant.

**62.** (D) Colony PCR
Colony PCR is a rapid and efficient technique used to screen bacterial colonies for the presence of a specific DNA insert. It allows researchers to verify both the occurrence and the size of the recombinant DNA directly from the culture without the need for time-consuming plasmid isolation steps. This method is ideal for high-throughput analysis of large numbers of potential clones.

**63.** (C) Bacterial cells lysed during the initial denaturation step.
In colony PCR, the initial template DNA is provided by the bacterial cells themselves. During the first step of the PCR program, a high-temperature denaturation phase causes the

sequence but cleave the strands at different positions. For example, SmaI and XmaI both target the same six-base sequence. However, SmaI produces blunt ends by cutting in the middle, while XmaI creates cohesive 5' overhangs. This difference in cleavage patterns makes them useful for distinct cloning applications.

**46.** (D) It integrates larger DNA inserts between 20 and 25 kilobases.
Bacteriophage lambda vectors are often preferred over standard plasmids when constructing genomic libraries. Their primary advantage lies in their ability to accommodate much larger pieces of foreign DNA, typically ranging between 20 and 25 kilobases. This increased capacity allows for the representation of an entire genome in a smaller number of clones, making the screening process more efficient.

**47.** (C) BAC (Bacterial Artificial Chromosome)
Bacterial Artificial Chromosomes are sophisticated vectors derived from the naturally occurring F-factor plasmid of E. coli. They utilize the F-factor's replication and partitioning systems to maintain a very low copy number, usually just one or two per cell. This stability allows for the cloning of large genomic inserts, up to 300 kilobases, with minimal risk of rearrangement or loss.

**48.** (B) CEN, TEL, ARS, and a selectable marker
For a Yeast Artificial Chromosome to function effectively as a linear chromosome in yeast cells, it must contain several essential genetic elements. These include a centromere (CEN) for segregation, telomeres (TEL) for protecting the ends of the molecule, and autonomous replicating sequences (ARS) to initiate DNA synthesis. Selectable markers are also included to allow for the identification of transformed cells.

**49.** (D) Plasmid containing lambda 'cos' sites for in vitro packaging.
A cosmid is a specialized type of hybrid cloning vector that combines properties of both plasmids and bacteriophage lambda. It contains a standard plasmid origin of replication and selectable markers, along with the lambda 'cos' sites. These sites allow the recombinant DNA to be packaged into lambda phage heads in vitro, facilitating the efficient delivery of large DNA inserts into bacteria.

**50.** (A) Electroporation
Electroporation is a physical method used to introduce foreign genetic material into cells. It involves applying short, high-voltage electrical pulses to a cell suspension, which creates temporary nanometer-sized pores in the plasma membrane. These transient openings allow DNA or other molecules to enter the cytoplasm. The membrane eventually reseals, leaving the cell transformed with the new genetic material for study.

**51.** (C) Gold or tungsten
The biolistic gene transfer method, also known as the gene gun, uses microcarriers to deliver DNA into target tissues. These microcarriers are typically made of dense, chemically inert materials such as gold or tungsten. After being coated with the DNA of interest, these particles are accelerated to high velocities, allowing them to penetrate the cell walls and membranes of the host.

**52.** (A) Calcium ions shield DNA and LPS negative charges for binding.
Treating bacterial cells with ice-cold calcium chloride is a common method for inducing chemical competence. The calcium ions serve to neutralize the negative charges on both the DNA phosphate backbone and the lipopolysaccharides of the cell membrane. This reduction in electrostatic repulsion allows the DNA to bind to the cell surface, facilitating its eventual uptake during a subsequent heat-shock step.

**53.** (D) Expression vector with prokaryotic promoter and RBS.
To successfully express a eukaryotic protein within a prokaryotic host like E. coli, a specialized expression vector is required. This vector must provide the necessary regulatory elements that the host cell's machinery can recognize, including a strong prokaryotic promoter and a ribosome binding site. These components ensure that the inserted eukaryotic gene is efficiently transcribed and translated into a functional protein.

**54.** (B) The capacity of the lambda phage head during in vitro packaging
The maximum size of DNA that can be cloned using a cosmid vector is primarily limited by the physical capacity of the bacteriophage lambda head. During the in vitro packaging process, the packaging machinery only accepts

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ions available to shield the negative charges on the DNA phosphate backbone. The resulting electrostatic repulsion between the strands makes it more difficult for mismatched or weak duplexes to remain stable, ensuring specific binding.

**91.** (B) $T_m = 4(G+C)+2(A+T)$
For short oligonucleotide sequences of less than 20 bases, the Wallace rule provides a simple and effective approximation of the melting temperature. The formula calculates $T_m$ by assigning four degrees Celsius for every Guanine or Cytosine base and two degrees for every Adenine or Thymine base. This method is commonly used for quick estimations during the design of PCR primers.

**92.** (A) It represents highly repetitive DNA sequences that find their partners quickly.
In Cot curve analysis, which measures the rate of DNA reassociation, a low $\text{Cot}_{\frac{1}{2}}$ value indicates that a DNA fraction consists of highly repetitive sequences. Because these sequences are present in many copies throughout the genome, they find their complementary partners much more quickly during the annealing process than unique or complex single-copy sequences, leading to faster reassociation rates.

**93.** (C) Lowers $T_m$ to allow hybridization at reduced temperatures.
Formamide is a chemical agent commonly added to hybridization buffers to lower the melting temperature of DNA duplexes. By disrupting the hydrogen bonds between base pairs, formamide allows hybridization to occur at significantly reduced temperatures. This is beneficial for maintaining the integrity of RNA or tissue samples that might otherwise be damaged by the high heat required for standard denaturation.

**94.** (D) High stringency allows multiple base-pair probe mismatches.
Hybridization stringency refers to the conditions that determine the specificity of probe binding. High temperature, low salt concentration, and the addition of formamide all increase stringency, meaning only perfectly matched duplexes remain stable. It is incorrect to state that high stringency allows for multiple base-pair mismatches; rather, such conditions are designed to prevent non-specific binding and ensure high accuracy.

**95.** (B) P-3, Q-4, R-1, S-2
DNA duplex stability is affected by several factors. High GC content increases stability due to triple hydrogen bonding. High salt concentrations stabilize the helix by shielding negative phosphate repulsion. Conversely, the presence of formamide or mismatched base pairs decreases stability by interfering with hydrogen bonds or disrupting the overall helix geometry, thereby lowering the melting temperature of the DNA molecule.

**96.** (B) Single-copy (unique) DNA
During a Cot curve experiment for eukaryotic genomic DNA, the reassociation process occurs in phases based on sequence complexity. The single-copy or unique DNA fraction, which contains the majority of functional genes, reassociates last. This is because these sequences are present only once per haploid genome, making the probability of a specific strand finding its complementary partner much lower than for repeats.

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**97.** (D) The increase in 260 nm UV absorbance during DNA denaturation.
The hyperchromic shift is a phenomenon observed during the denaturation of double-stranded DNA. As the hydrogen bonds between base pairs break and the two strands separate into single strands, there is a significant increase in the absorbance of ultraviolet light at 260 nm. This change occurs because the stacked bases in a double helix absorb less light than unstacked bases.

**98.** (A) PCR primer design
Accurate calculation of the melting temperature for oligonucleotides is most critical during the design of PCR primers. The annealing temperature of a PCR cycle must be optimized based on the $T_m$ of the primer pair to ensure specific and efficient amplification of the target DNA. Incorrect $T_m$ calculations can lead to poor yields, non-specific products, or complete failure of the reaction.

**99.** (B) Decreases the melting temperature ($T_m$) by disrupting hydrogen bonds.
Chemical denaturants such as urea and formamide decrease the stability of a DNA duplex. They achieve this by competing for and disrupting the hydrogen bonds between complementary nitrogenous bases. As a result, the melting temperature of the DNA molecule is

bacterial cells to lyse, releasing their plasmid and chromosomal DNA into the reaction mixture. This released DNA then serves as the template for the polymerase to amplify the target sequence of interest.

**64.** (B) Ampicillin sensitive, Tetracycline resistant
When a cloning strategy involves the insertional inactivation of the ampicillin resistance gene while leaving the tetracycline resistance gene intact, the phenotypes of the cells change. A successful recombinant will be sensitive to ampicillin because that gene is disrupted. However, it will remain resistant to tetracycline, allowing researchers to select for these specific clones by comparing growth on different antibiotic plates.

**65.** (D) P-3, Q-4, R-1, S-2
Recombinant screening involves several key biochemical components. X-gal serves as a chromogenic substrate that turns blue when cleaved. IPTG acts as an inducer by binding to the lac repressor, which normally prevents transcription. Beta-galactosidase is the enzyme responsible for cleaving lactose analogs. The lac repressor protein regulates the system by binding to the operator to block the synthesis of the enzyme.

**66.** (A) Small in-frame insertions that do not abolish enzyme activity.
A false positive in blue-white screening occurs when a colony appears blue despite containing an insert. This can happen if the inserted DNA is very small and stays in-frame with the lacZ-alpha sequence. In such cases, the resulting fusion protein may still retain enough enzymatic activity to cleave X-gal, leading to a blue color that mistakenly suggests a non-recombinant plasmid.

**67.** (C) It must carry a lacZ mutation (e.g. $\Delta M_{15}$) deleting the alpha part.
For alpha-complementation to work as a screening tool, the host E. coli strain must possess a specific genetic mutation, such as the $\Delta M_{15}$ deletion. This mutation removes the N-terminal alpha-portion of the lacZ gene from the bacterial genome. The remaining part of the gene produces an inactive omega-peptide, which can only become functional when complemented by the plasmid-encoded alpha-peptide.

**68.** (A) Amplification of a short fragment from flanking vector DNA.
When vector-specific primers that flank the multiple cloning site are used in colony PCR on a non-recombinant colony, the polymerase amplifies the empty region between them. Because no foreign DNA has been inserted, the resulting PCR product will be a short fragment consisting only of the original vector sequences. This provides a clear size distinction compared to recombinant clones.

**69.** (D) Nick translation
Nick translation is a technique used to label DNA probes for hybridization assays. It begins with the action of DNase I, which introduces random single-stranded nicks into the double-stranded DNA template. DNA polymerase I then utilizes its 5' to 3' exonuclease activity to remove nucleotides from the nick site while simultaneously synthesizing a new, labeled strand using its polymerase activity.

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**70.** (B) Generates higher specific activity probes from less template.
Random primer labeling is often preferred because it can generate probes with very high specific activity using only a small amount of template DNA. This method uses short hexanucleotides that anneal randomly to denatured single-stranded DNA, serving as primers for a polymerase like the Klenow fragment. This results in the efficient synthesis of highly labeled, sensitive probes for molecular detection.

**71.** (C) $T_4$ Polynucleotide kinase
The labeling of the 5' end of a DNA molecule with a radioactive phosphate group requires the enzyme $T_4$ Polynucleotide kinase. Before the labeling reaction, the existing 5' phosphate is typically removed by alkaline phosphatase. The kinase then transfers the gamma-phosphate from a labeled ATP molecule to the 5'-hydroxyl group of the DNA, creating a highly specific radioactive probe for assays.

**72.** (D) Non-radioactive probes require stringent isotope disposal protocols.
Radioactive and non-radioactive probes are compared based on their safety, sensitivity, and shelf life. It is incorrect to state that non-radioactive probes, such as those labeled with biotin or digoxigenin, emit radiation. In fact,

these probes are designed specifically to avoid the hazards of ionizing radiation and the strict disposal protocols required for isotopes like Phosphorus-32 used in laboratories.

**73.** (A) Biotin
Biotin is a small molecule frequently incorporated into nucleotides for non-radioactive probe labeling. Once the biotinylated probe hybridizes to its target, it can be detected with high sensitivity using the protein avidin or streptavidin, which has an extremely high affinity for biotin. These proteins are often conjugated to reporter enzymes like horseradish peroxidase to produce a measurable signal in assays.

**74.** (B) Lacks 5'-3' exonuclease to prevent new probe degradation.
The Klenow fragment is preferred for random primer labeling because it lacks the 5' to 3' exonuclease activity found in the full DNA polymerase I holoenzyme. This deficiency is crucial as it prevents the enzyme from degrading the newly synthesized, labeled probe strands during the reaction. By only possessing polymerase activity, the Klenow fragment ensures the efficient and stable production of probes.

**75.** (A) I, II and III
Various enzymatic methods are used to label DNA probes. Nick translation requires DNase I to create nicks, while random primer labeling utilizes hexanucleotides to initiate synthesis. For end labeling, terminal deoxynucleotidyl transferase is used at the 3' end, and a combination of alkaline phosphatase and polynucleotide kinase is used at the 5' end. These techniques allow for different labeling strategies.

**76.** (A) Detection via anti-DIG antibodies coupled to reporter enzymes.
Digoxigenin is a steroid found exclusively in digitalis plants, making it an excellent hapten for non-radioactive labeling since it does not occur naturally in animal or plant tissues. DIG-labeled probes are detected using specific anti-digoxigenin antibodies that are coupled to reporter enzymes. These enzymes then produce a chemiluminescent or colorimetric signal upon the addition of a suitable substrate during diagnostics.

**77.** (C) Sufficient length (18-30 bases) and appropriate GC content.
When designing synthetic oligonucleotide probes, it is essential to ensure high specificity and stable hybridization. This is achieved by selecting a sequence of sufficient length, typically between 18 and 30 bases, and maintaining an appropriate GC content. These factors help to establish an optimal melting temperature and prevent non-specific binding to unrelated genomic sequences during the hybridization procedure.

**78.** (D) Ser65-Tyr66-Gly67
The maturation of the Green Fluorescent Protein chromophore involves a spontaneous internal reaction of a specific tripeptide sequence. This sequence consists of the amino acid residues Serine 65, Tyrosine 66, and Glycine 67. These three residues undergo a process of cyclization and subsequent oxidation, which creates the structure responsible for the protein's characteristic green fluorescence when exposed to light.

**79.** (C) Molecular oxygen ($O_2$)
The formation of the mature chromophore in wild-type Green Fluorescent Protein is an autocatalytic process that does not require external enzymes. However, it is strictly dependent on the presence of molecular oxygen. Oxygen is required for the oxidation step of the cyclized tripeptide, which completes the chemical transformation necessary for the protein to become fluorescent within the host cell.

**80.** (B) Point mutations shift excitation peaks and increase intensity.
Enhanced variants of the Green Fluorescent Protein, such as EGFP, have been developed through specific point mutations in the amino acid sequence. These changes primarily serve to shift the excitation peaks to more favorable wavelengths and significantly increase the intensity of the fluorescence signal. Such engineering makes these variants much more useful for sensitive imaging applications in various biological systems.

**81.** (D) Beta-barrel
The Green Fluorescent Protein possesses a highly stable and unique three-dimensional structure known as a beta-barrel. This motif consists of eleven anti-parallel beta-strands that form a cylindrical protective cage around the

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central alpha-helix. This structural arrangement is crucial because it shields the internal chromophore from the external environment, ensuring the protein remains fluorescent under a variety of cellular conditions.

**82.** (A) Quantifying precise enzymatic reaction kinetics in vitro
While Green Fluorescent Protein is a powerful tool for visualizing protein localization and tracking gene expression in live cells, it is not well-suited for quantifying precise enzymatic reaction kinetics in vitro. Enzymatic studies typically require more direct biochemical measurements of substrates and products, whereas GFP fluorescence provides a qualitative or semi-quantitative marker of protein presence rather than activity.

**83.** (C) Non-toxic visible tag for tracking subcellular localization.
GFP is frequently fused to the N- or C-terminus of a target protein because it acts as a non-toxic, visible tag. This fusion allows researchers to track the subcellular localization and movement of the protein in real-time within living cells. By maintaining the protein's natural behavior while adding a fluorescent marker, cell biologists can observe dynamic biological processes directly.

**84.** (A) Amino acid substitutions altering the chromophore environment.
The development of a diverse palette of fluorescent proteins, such as Yellow and Blue Fluorescent Proteins, is achieved through specific amino acid substitutions. These mutations alter the local chemical environment surrounding the chromophore, which in turn shifts its excitation and emission spectra. This allows for the simultaneous visualization of multiple different proteins or cellular structures using distinct colors during imaging.

**85.** (A) Both A and R are true and R is the correct explanation of A.
Fluorescence Resonance Energy Transfer can be used with GFP variants to study protein-protein interactions. This occurs when two proteins, each fused to a different colored variant, come into close proximity. If the emission spectrum of the donor variant overlaps with the excitation spectrum of the acceptor, energy is transferred non-radiatively. Measuring this change in fluorescence confirms the physical interaction.

**86.** (B) Tissue absorbance and scattering of emission wavelengths.
Using standard Green Fluorescent Protein for in vivo imaging in thick tissues presents significant challenges. The primary limitation is that biological tissues absorb and scatter light, particularly at the shorter wavelengths where GFP emits. This results in reduced signal penetration and poor resolution when trying to visualize structures deep within an organism, often necessitating the use of red-shifted fluorescent proteins.

**87.** (C) Use spectral variants like EGFP and mCherry for distinction.
To visualize the simultaneous expression of two different genes within the same cell, researchers utilize spectral variants of fluorescent proteins. By using distinct colors, such as EGFP for one gene and mCherry for another, the signals can be separated based on their unique emission wavelengths. This multiplexing approach allows for the direct observation of how different genes interact and localize.

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**88.** (D) Temperature where 50% of the DNA duplexes are dissociated.
The melting temperature of a DNA molecule is a fundamental thermodynamic property. It is defined as the specific temperature at which 50% of the DNA duplexes in a solution have dissociated into individual single strands. This value is critical for various molecular techniques, including PCR and hybridization, as it dictates the stability of the double-helical structure under specific experimental conditions.

**89.** (A) High proportion of Guanine and Cytosine
The melting temperature of a DNA duplex is directly influenced by its nitrogenous base composition. A higher proportion of Guanine and Cytosine residues increases the $T_m$ because these pairs are held together by three hydrogen bonds, compared to only two for Adenine and Thymine. This extra bond provides greater thermal stability to the helix, requiring more energy to denature the strands.

**90.** (B) Increases stringency via electrostatic repulsion of backbones.
In nucleic acid hybridization, lowering the concentration of monovalent salts, like sodium ions, increases the stringency of the reaction. This occurs because there are fewer positive

reliably generate DNA fragments with known ends for ligation into various vector systems.

**37.** (D) Different species enzymes recognizing and cutting identical sequences.
Isoschizomers are restriction endonucleases that are isolated from different bacterial species but recognize and cleave the exact same DNA sequence. While they target identical sites, they may differ in their sensitivity to DNA methylation or their reaction conditions. These enzymes are useful in molecular biology when a specific recognition sequence needs to be targeted using alternative enzymatic sources or properties.

**38.** (B) It requires ATP as a cofactor to catalyze phosphodiester bond formation.
$T_4$ DNA ligase is a critical enzyme used to join DNA fragments by catalyzing the formation of phosphodiester bonds between adjacent 3'-hydroxyl and 5'-phosphate termini. This process requires ATP as an essential cofactor to energize the reaction. Unlike some other ligases, the $T_4$ variant is highly versatile, capable of joining both cohesive (sticky) and blunt-ended DNA molecules during cloning.

**39.** (C) Removes 5' phosphate groups from vectors to prevent selF$^-$ligation.
Alkaline Phosphatase is frequently employed during the preparation of vector DNA for molecular cloning. Its primary function is to remove the 5' phosphate groups from the ends of a linearized plasmid. This modification prevents the vector from recircularizing through selF$^-$ ligation during the subsequent ligation step. Consequently, the efficiency of inserting foreign DNA fragments into the vector is significantly increased.

**40.** (D) Klenow fragment of DNA polymerase I
The Klenow fragment is a modified version of DNA polymerase I that retains its 5' to 3' polymerase and 3' to 5' exonuclease activities. It is widely used in molecular biology to create blunt ends on DNA fragments. It can fill in recessed 5' overhangs by adding nucleotides or remove protruding 3' overhangs, ensuring the termini are suitable for ligation.

**41.** (A) $S_1$ nuclease - Cleaves double-stranded DNA specifically
$S_1$ nuclease is an enzyme characterized by its high specificity for single-stranded nucleic acids. It effectively degrades single-stranded DNA or RNA into mononucleotides, while leaving double-stranded regions relatively intact. Therefore, stating that it cleaves double-stranded DNA specifically is incorrect. Other enzymes, such as reverse transcriptase and polynucleotide kinase, are correctly identified by their roles in synthesizing DNA or transferring phosphate groups.

**42.** (B) The relaxation of sequence specificity under suboptimal buffer conditions
Star activity refers to a phenomenon where restriction endonucleases lose their strict sequence specificity and begin to cleave DNA at sites that differ slightly from their canonical recognition sequence. This typically occurs under suboptimal laboratory conditions, such as high glycerol concentration, low ionic strength, or extreme pH values. Researchers must carefully control buffer environments to prevent this unintended non-specific digestion.

**43.** (B) Escherichia genus, coli species, RY13 strain, first enzyme isolated.
The nomenclature for restriction enzymes follows a standardized system based on the source organism. For EcoRI, the 'E' represents the genus Escherichia, and 'co' stands for the species coli. The 'R' designates the specific strain, RY13, from which the enzyme was first isolated. The Roman numeral 'I' indicates that it was the first restriction enzyme identified in that strain.

**44.** (A) It adds nucleotides to the 3' end of DNA without requiring a template.
Terminal deoxynucleotidyl transferase is a unique DNA polymerase that functions independently of a DNA or RNA template. Unlike standard polymerases, it adds deoxynucleotides directly to the 3'-hydroxyl terminus of a DNA molecule. This capability is frequently utilized in molecular biology for homopolymer tailing, allowing for the addition of specific sequences to the ends of DNA fragments for cloning or labeling.

**45.** (A) Both A and R are true and R is the correct explanation of A.
Neoschizomers are a specific subset of isoschizomers that recognize the identical DNA

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lowered, allowing the two strands to separate at a temperature that would normally maintain the double-helical structure in pure water.

**100.** (A) Cot-1 DNA or blocking reagents
In Fluorescence In Situ Hybridization, preventing non-specific binding is crucial for clear results. Researchers often use Cot-1 DNA, which is enriched for repetitive sequences, to block these regions in the genome and prevent the probe from binding to offF-target sites. This increases the signal-to-noise ratio by ensuring that the fluorescent probe only hybridizes to its specific complementary target sequence within the cell.

**101.** (B) Identifying chromosomal translocations and structural rearrangements.
Chromosome painting is a clinical cytogenetic technique used to identify structural abnormalities in the genome. By using fluorescently labeled probes that cover entire chromosomes, researchers can visualize chromosomal translocations and other rearrangements under a microscope. This method is particularly useful for detecting complex changes that might be missed by traditional banding techniques, helping in the diagnosis of various genetic disorders.

**102.** (D) DAPI (4',6-diamidino-2-phenylindole)
DAPI is a fluorescent stain that binds strongly to A-T rich regions in DNA and is frequently used as a counterstain in FISH experiments. Its primary role is to visualize the overall nuclear morphology by causing the cell nuclei to glow blue under ultraviolet light. This allows researchers to locate the cells and provide context for the specific signals from probes.

**103.** (D) Target DNA must stay double-stranded during the entire protocol.
FISH methodology involves several steps to visualize specific sequences. It is incorrect to state that target DNA must stay double-stranded; in fact, both the probe and the target DNA must be denatured into single strands for hybridization to occur. The protocol also requires fixed and permeabilized cells, specific fluorophore labels, and a fluorescence microscope for the detection of the signals.

**104.** (C) Spectral Karyotyping (SKY) or Multiplex FISH (M-FISH).
Spectral Karyotyping and Multiplex FISH are advanced techniques designed to visualize the entire human karyotype simultaneously. By using a combination of five different fluorochromes to label each chromosome with a unique spectral signature, every chromosome pair can be identified by its color. This approach is highly effective for identifying complex chromosomal translocations and marker chromosomes in clinical and research settings.

**105.** (A) Both A and R are true and R is the correct explanation of A.
In Situ Hybridization is a powerful technique for detecting specific nucleic acid sequences within tissue samples. It can be used to identify viral RNA in host biopsies by designing probes that hybridize specifically to the viral transcripts. Because the process is performed while maintaining the cellular architecture, it allows researchers to pinpoint exactly which cells are infected by the virus.

**106.** (A) Digesting proteins to facilitate probe access to nucleic acids.
During an In Situ Hybridization protocol, the tissue sample is typically treated with Proteinase K. This enzyme digests cellular proteins that might otherwise physically block the access of the probe to its target nucleic acid. By partially breaking down the protein matrix, the protease enhances the penetration of the probe into the cells, leading to more efficient and sensitive hybridization results.

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**107.** (B) Enables multiplexing via spectrally distinct fluorophore labels.
Fluorescence In Situ Hybridization offers a major advantage over traditional colorimetric methods through its ability to perform multiplexing. By using multiple spectrally distinct fluorophore labels, researchers can simultaneously detect and distinguish several different nucleic acid targets within a single cell or tissue section. This capability provides a much more comprehensive view of gene expression or chromosomal arrangement in biological studies.

**108.** (B) Centromere of chromosome 17 (CEP17) control probe.
A diagnostic HER2 FISH assay is used to determine if a patient's breast cancer cells have extra copies of the HER2 gene. To ensure accuracy, the assay includes a control probe that targets the centromere of chromosome 17. By comparing the number of HER2 signals to

the number of control signals, clinicians can distinguish between gene amplification and chromosome aneuploidy.

**109.** (A) Maps genes on extended single DNA fibers for high resolution.
Fiber-FISH is a high-resolution variant of the standard hybridization technique. It involves mapping genes or sequences onto individual DNA fibers that have been mechanically stretched and immobilized on a glass slide. This method provides much greater spatial resolution than metaphase or interphase FISH, allowing for the precise measurement of distances between probes and the detection of small structural variations.

**110.** (B) Silver halide reduction in emulsion by ionizing radiation.
Autoradiography is a technique used to detect radioactive substances in a sample. The process relies on ionizing radiation emitted by an isotope, which strikes silver halide crystals in a photographic emulsion. This interaction reduces the silver ions to metallic silver atoms, forming a latent image. When the film is developed, these darkened areas reveal the location and intensity of the radioactivity.

**111.** (B) 14.3 days
Phosphorus-32 is a high-energy beta-emitting radioisotope frequently used in molecular biology for labeling nucleic acids. It has a relatively short biological halF-life of approximately 14.3 days. Due to this rapid decay, researchers must complete their experiments and detection steps quickly, and the isotope requires careful handling and specialized shielding to protect personnel from radiation exposure in the laboratory.

**112.** (C) 35S
Sulfur-35 is the most commonly used radioisotope for labeling proteins during biological experiments. It is typically incorporated into proteins by growing cells in media containing labeled amino acids like methionine or cysteine. The emission of low-energy beta particles from the sulfur atoms allows researchers to track protein synthesis and degradation in pulse-chase experiments with high sensitivity and good resolution.

**113.** (C) 12.3 years
Tritium, or Hydrogen-3, is a radioisotope that emits very low-energy beta radiation. It is widely used in biological research for labeling a variety of organic molecules. One of its defining characteristics is its long halF-life of approximately 12.3 years. This longevity makes it suitable for long-term metabolic studies, although its weak emission requires specialized detection methods to visualize.

**114.** (B) $^{32}P > ^{35}S > ^{14}C > ^{3}H$
Radioisotopes used in the laboratory differ in the energy of the beta particles they emit. Phosphorus-32 is the strongest, providing high-energy radiation that requires Plexiglas shielding. Sulfur-35 and Carbon-14 follow with significantly lower energies, which can often be managed with standard lab equipment. Tritium is the weakest emitter, with beta particles that are easily absorbed by a few microns of water.

**115.** (A) P-2, Q-1, R-3, S-4
Different radioisotopes are selected based on the specific molecular biology application. Phosphorus-32 is primarily used for end-labeling DNA probes due to its high energy. Sulfur-35 is ideal for labeling amino acids in proteins. Tritium is used for high-resolution autoradiography because its weak emission stays localized. Carbon-14 is frequently employed for long-term metabolic tracing and historical dating of biological samples.

**116.** (B) Converts beta particles to light to increase detection sensitivity.
Intensifying screens are used in autoradiography to enhance the detection of high-energy beta particles from Phosphorus-32. The screen contains a phosphor that absorbs the radiation and converts it into visible light, which then exposes the X-ray film more efficiently than the particles alone. Performing this at -70°C helps to stabilize the latent image and further increase the sensitivity of detection.

**117.** (D) Weak beta particles cannot penetrate film coating effectively.
A major limitation of using Tritium in standard X-ray film autoradiography is the extremely low energy of its beta emissions. These weak particles often lack the energy to penetrate the protective coating of the film or the sample itself. Consequently, Tritium signals are difficult to detect using traditional methods, often requiring the use of specialized emulsions or liquid scintillation.

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**118.** (C) Thick Plexiglas or acrylic shielding
Protecting laboratory personnel from the high-energy beta radiation emitted by Phosphorus-32 requires specific materials. Thick Plexiglas or acrylic shielding is recommended because these materials effectively stop the beta particles while minimizing the production of secondary X-rays, known as Bremsstrahlung. Using heavy metals like lead is avoided for primary shielding as they can actually increase the production of these X-rays.

**119.** (A) A latent image consisting of metallic silver atoms in the crystal.
When ionizing radiation from a radioactive sample strikes a silver bromide crystal in a photographic emulsion, it initiates a chemical change. This process leads to the formation of a latent image, which consists of small clusters of metallic silver atoms within the crystal lattice. These clusters act as centers for the subsequent development process, which amplifies the signal into visible spots.

**120.** (A) Southern blotting
Southern blotting is a cornerstone technique in molecular biology used to identify specific DNA sequences within a complex mixture. The process involves separating DNA fragments by size using gel electrophoresis, transferring them onto a stable membrane, and then hybridizing the membrane with a labeled probe. This allows researchers to detect the presence and size of a target gene with high specificity.

**121.** (C) Northern detects RNA; Western detects proteins.
Northern and Western blotting are fundamental techniques that target different classes of biological macromolecules. Northern blotting is specifically designed for the detection and quantification of RNA sequences, often used to study gene expression levels. In contrast, Western blotting is used to identify and analyze specific proteins within a sample, typically utilizing antibodies as probes for detection after electrophoresis.

**122.** (C) Depurinates DNA to fragment it for efficient membrane transfer.
Before transferring DNA from an agarose gel to a membrane during Southern blotting, the gel is often treated with a dilute acid like hydrochloric acid. This step serves to depurinate the DNA, which facilitates the subsequent cleavage of the strands in the alkaline transfer buffer. This fragmentation is necessary to ensure that large DNA molecules can efficiently migrate out of the gel.

**123.** (B) DNA-binding proteins
Southwestern blotting is a specialized hybrid technique used to identify proteins that have a specific affinity for DNA sequences. In this method, proteins are first separated by SDS-PAGE and then transferred to a membrane. The membrane is subsequently incubated with a labeled DNA probe. This allows researchers to detect and characterize DNA-binding proteins, such as transcription factors, based on their binding activity.

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**124.** (D) Nitrocellulose binds DNA covalently without baking or UV.
Various membrane supports are used for blotting, each with distinct properties. Nitrocellulose is popular but fragile, while nylon is stronger and more durable. PVDF requires methanol pre-wetting to become functional. It is incorrect to say that nitrocellulose binds DNA covalently without further treatment; in fact, the DNA must be permanently fixed to the membrane using heat baking or ultraviolet crosslinking.

**125.** (D) I, II, and III
Western blotting involves several key steps for protein detection. Primary antibodies are used to bind specifically to the target protein on the membrane. Secondary antibodies, which are often conjugated to an enzyme like horseradish peroxidase, then bind to the primary antibody to amplify the signal. Blocking agents like milk or bovine serum albumin are essential for preventing non-specific antibody binding.

**126.** (B) To covalently crosslink the nucleic acids to the membrane matrix
Once DNA has been transferred from a gel to a membrane during Southern blotting, it must be permanently attached to the surface. This is achieved by either baking the membrane at 80 degrees Celsius or exposing it to ultraviolet radiation. These treatments create covalent crosslinks between the nucleic acids and the membrane matrix, ensuring the DNA remains fixed during hybridization.

being synthesized, enabling real-time recording of nucleotide incorporation events.

**145.** (A) I, II and IV
Next-generation sequencing encompasses diverse technologies. Illumina typically produces short reads of a few hundred base pairs, while Nanopore sequencing is unique because it can analyze DNA directly without the need for prior amplification. PacBio utilizes Zero-Mode Waveguides to achieve single-molecule detection. It is incorrect to say that Ion Torrent uses fluorescent nucleotides; it instead detects changes in pH levels during synthesis.

**146.** (A) Electrostatic interaction between charges and stationary phase.
Ion-exchange chromatography is a powerful technique for protein purification that separates molecules based on their surface charge. The fundamental principle involves the electrostatic interaction between the charged groups on the protein and the oppositely charged ligands attached to the stationary phase resin. By adjusting the pH or ionic strength of the buffer, proteins can be selectively bound and then eluted.

**147.** (A) Cation-exchange resin
The net charge of a protein depends on the relationship between the buffer pH and the protein's isoelectric point. At a pH of 7.0, a protein with a pI of 9.5 will have a net positive charge because the environment is more acidic than its pI. Therefore, it will bind most effectively to a cation-exchange resin, which carries negative charges.

**148.** (B) Largest molecules as they are excluded into void volume.
Gel filtration chromatography, also known as size-exclusion chromatography, separates molecules based on their physical dimensions. The stationary phase consists of porous beads. Large molecules are too big to enter these pores and are therefore excluded, traveling only through the void volume between the beads. As a result, the largest molecules elute first from the column, followed by smaller molecules that enter the matrix.

**149.** (B) His-tag
The histidine-tag (His-tag) is one of the most widely used affinity tags in recombinant protein purification. It consists of a string of six to ten histidine residues that are fused to the target protein. These residues form strong coordinate covalent bonds with immobilized metal ions, such as Nickel or Cobalt, which are attached to a resin, allowing for highly specific purification.

**150.** (B) By washing with a buffer containing a high concentration of imidazole
To recover a His-tagged protein from a Nickel-NTA affinity column, researchers typically use a buffer containing a high concentration of imidazole. Imidazole has a chemical structure similar to the side chain of histidine and acts as a competitive binder. By outcompeting the histidine residues for the limited number of binding sites on the nickel ions, the imidazole causes the protein to elute.

**151.** (D) Protein A - High pH buffer (e.g., pH 10.0)
Different affinity tags require specific agents for elution. GST-tags are eluted using reduced glutathione, and Maltose Binding Protein is eluted with maltose. For His-tagged proteins, imidazole is used. It is incorrect to state that Protein A is eluted with a high pH buffer; instead, a low pH buffer, typically around pH 3.0, is used to disrupt the strong antibody-binding interaction.

**152.** (B) Salt ions compete with negative proteins for resin sites.
In anion-exchange chromatography, proteins with a net negative charge bind to the positively charged resin. The resolution of these proteins is achieved by applying a salt gradient, such as increasing concentrations of sodium chloride. The salt ions compete directly with the negatively charged protein surface for the binding sites on the resin. As the salt concentration increases, proteins with weaker charges elute first.

**153.** (A) Enhances solubility of the expressed fusion protein.
While the primary use of a Glutathione S-transferase tag is to facilitate protein purification via affinity chromatography, it offers additional benefits. A significant advantage is that the GST-tag often enhances the solubility of the recombinant fusion protein when expressed in E. coli. This can prevent the formation of insoluble inclusion bodies, making it easier to recover functional and correctly folded proteins.

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**136.** (A) It requires hazardous chemicals and is hard to automate.
Although it was once a primary method for DNA sequencing, the Maxam-Gilbert technique has largely been replaced by the Sanger method and next-generation technologies. This shift occurred because the Maxam-Gilbert protocol requires the use of hazardous chemicals, such as hydrazine and dimethyl sulfate. Additionally, the process is difficult to automate and scale up, making it less practical for large sequencing projects.

**137.** (B) Illumina
The Illumina sequencing platform is the most widely used next-generation technology and operates on the principle of sequencing by synthesis. It utilizes a special type of nucleotide called a reversible dye-terminator. These nucleotides have a fluorescent tag that also acts as a terminator, which can be chemically removed after each base addition, allowing the next nucleotide to be incorporated in subsequent cycles.

**138.** (B) By detecting the release of a hydrogen ion ($H^+$) which causes a micro-pH change
Ion Torrent sequencing uses a unique semiconductor-based detection system to read DNA. When a nucleotide is incorporated into a growing DNA strand by a polymerase, a hydrogen ion is naturally released as a byproduct. The system detects the resulting microscopic change in pH within a small well on the chip, converting this chemical signal directly into digital information for sequence analysis.

**139.** (C) Pacific Biosciences (PacBio)
Pacific Biosciences developed a single-molecule real-time sequencing technology known as SMRT sequencing. This method is classified as a long-read technology because it can generate continuous sequences that are tens of kilobases in length. By observing a single DNA polymerase molecule in real time as it incorporates fluorescently labeled nucleotides, it provides a powerful tool for resolving complex genomic structures and repeats.

**140.** (B) Recording current disruptions as DNA threads through a pore.
Oxford Nanopore sequencing is a revolutionary technology that measures changes in electrical current to read DNA. As a single-stranded DNA molecule is threaded through a tiny protein pore in a membrane, each base disrupts the steady ionic flow in a characteristic way. These disruptions are recorded as a signal, which is then translated into the DNA sequence, allowing for portable sequencing.

**141.** (A) Generating localized clonal clusters for signal intensity.
Bridge amplification is a critical step in the Illumina sequencing workflow that occurs on the surface of a flow cell. This process uses localized PCR to generate millions of clonal clusters from individual DNA fragments. By creating these dense clusters of identical sequences, the platform ensures that the fluorescent signal from each incorporated nucleotide is bright enough to be accurately detected by imaging.

**142.** (C) Indels resulting from non-linear signal intensity.
In sequencing methods like Ion Torrent and Pyrosequencing, homopolymer regions—where the same base is repeated multiple times—can lead to errors. Because these platforms measure signal intensity to determine the number of bases incorporated, the relationship between signal and length can become non-linear for long repeats. This often results in insertion or deletion errors (indels), which are a characteristic limitation of these technologies.

**143.** (B) Long-read is superior for resolving complex genomic repeats and structural variants.
Short-read technologies like Illumina offer extremely high throughput and accuracy but struggle with large genomic repeats. In contrast, long-read platforms such as PacBio and Nanopore are superior for resolving complex structural variants and assembling genomes because they can span long repetitive regions. While each has its strengths, long-reads are particularly valuable for filling gaps in genomic maps that short-reads cannot bridge.

**144.** (B) Immobilized at the bottom of a Zero-Mode Waveguide (ZMW)
In the Pacific Biosciences sequencing system, the DNA polymerase enzyme is not floating in the solution. Instead, it is specifically immobilized at the bottom of a Zero-Mode Waveguide, which is a tiny nanophotonic structure. This immobilization allows the system to focus its detection on a single molecule of DNA as it is

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**199.** (B) Obtaining de novo peptide sequence via precursor fragmentation.
Tandem Mass Spectrometry is an essential tool for obtaining de novo sequence information from peptides. In this method, a specific precursor ion is selected and then fragmented, usually through collisions with an inert gas. The resulting fragment ions are then analyzed in a second stage of mass spectrometry. This fragmentation pattern reveals the amino acid sequence and the location of any modifications on the peptide.

**200.** (A) Mass spectrometry directly sequences the mRNA corresponding to the protein.
Mass spectrometry-based proteomics is a powerful field that identifies proteins and their modifications. Trypsin is used for specific cleavage, and MALDI is often paired with TOF analyzers for mass measurement. It is incorrect to state that mass spectrometry directly sequences the mRNA; instead, the technology analyzes the peptides themselves, and bioinformatic tools are then used to match these back to genomic data.

**201.** (A) The transducer
A biosensor consists of a biological recognition element and a physical component known as the transducer. The transducer is the part of the system responsible for converting the specific biological interaction, such as an antibody binding to an antigen, into a measurable electrical, optical, or thermal signal. This signal is then processed and quantified to determine the concentration of the target analyte.

**202.** (C) The refractive index near a sensor surface upon analyte binding
Surface Plasmon Resonance biosensors are used to monitor molecular interactions in real-time without the need for labels. The technique works by detecting changes in the refractive index very close to the sensor surface. When an analyte molecule binds to a ligand immobilized on the surface, it alters the mass and the local refractive index, which causes a measurable shift in the resonance angle.

**203.** (B) The association ($k_{on}$) and dissociation ($k_{off}$) rate constants of the interaction
A sensogram generated by an SPR experiment provides a wealth of information about how two molecules interact. By analyzing the shape of the binding and dissociation curves, researchers can directly determine the kinetic rate constants, including the association rate ($k_{on}$) and the dissociation rate ($k_{off}$). These values are then used to calculate the overall binding affinity or equilibrium dissociation constant of the complex.

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**204.** (B) Glucose oxidase
In a standard electrochemical glucose biosensor, the bioreceptor is the enzyme glucose oxidase. This enzyme specifically recognizes and catalyzes the oxidation of glucose molecules in the sample. This chemical reaction produces gluconolactone and hydrogen peroxide, while transferring electrons to an electrode. The resulting current is directly proportional to the concentration of glucose, allowing for the rapid monitoring of blood sugar levels.

**205.** (A) Both A and R are true and R is the correct explanation of A.
Quantum dots are highly effective for multiplexed cellular imaging because of their unique optical properties. They possess narrow and size-tunable emission spectra, which allow multiple colors to be clearly distinguished. Additionally, they have a broad excitation spectrum, meaning several different colored dots can be excited using a single light source. This makes them superior to traditional dyes for complex multi-target experiments.

**206.** (B) They are highly toxic and degrade rapidly in aqueous solutions.
Gold nanoparticles are widely used in nanodiagnostics due to their unique properties, such as localized surface plasmon resonance and ease of functionalization with DNA. They undergo a noticeable color change upon aggregation, which is useful for sensing. It is incorrect to claim they are highly toxic or degrade rapidly; in fact, they are known for their high biocompatibility and excellent stability in solution.

**207.** (A) They are highly resistant to photobleaching.
One of the most significant advantages of quantum dots over traditional organic fluorophores is their extreme resistance to photobleaching. Unlike standard dyes, which quickly lose their ability to fluoresce when exposed to light, quantum dots can withstand continuous illumination for extended periods. This property makes them ideal for long-term imaging studies and tracking biological processes over several days in living cells.

**154.** (B) Restriction endonucleases and sequence-specific hybridization probes
Restriction Fragment Length Polymorphism analysis is a classical molecular method used to detect genetic variation. It fundamentally relies on the use of restriction endonucleases to digest genomic DNA into fragments. These fragments are then separated by size and analyzed using sequence-specific hybridization probes on a Southern blot. The resulting banding patterns reveal differences in the DNA sequence at the restriction sites.

**155.** (A) Both A and R are true and R is the correct explanation of A.
RFLP is considered a co-dominant marker system because it can simultaneously detect and distinguish between different alleles at a single locus. In a heterozygous individual, both alleles will produce distinct banding patterns on a blot, allowing them to be identified. This is in contrast to dominant markers, which cannot differentiate between a homozygote and a heterozygote for the dominant trait.

**156.** (B) PCR-based method using short arbitrary primers without sequence knowledge.
RAPD is a PCR-based technique that differs significantly from RFLP. Unlike RFLP, which requires large amounts of DNA and sequence-specific probes, RAPD utilizes short, arbitrary primers that anneal to random locations in the genome. It does not require any prior knowledge of the target DNA sequence, making it a quick and convenient method for initial genetic screening and fingerprinting.

**157.** (C) Low reproducibility across different laboratories and PCR platforms
A major disadvantage of the RAPD marker system is its low reproducibility. Because the technique relies on the annealing of short primers at low temperatures, the resulting banding patterns are highly sensitive to even minor changes in PCR conditions, such as the concentration of magnesium or the specific brand of thermal cycler. This makes it difficult to compare results between different laboratories.

**158.** (B) RFLP and PCR
Amplified Fragment Length Polymorphism is a versatile molecular marker system that combines the principles of both RFLP and PCR. It involves the restriction digestion of genomic DNA, the ligation of specific adapters to the fragments, and the subsequent selective amplification of these fragments using PCR. This approach provides the high sensitivity of PCR while maintaining the robust nature of restriction analysis.

**159.** (B) Cohesive ends generated by restriction enzyme digestion.
In the AFLP protocol, the initial step involves digesting genomic DNA with a combination of two different restriction enzymes. Following digestion, double-stranded DNA adapters are ligated specifically to the cohesive ends of the resulting fragments. These adapters serve as known sequences for the subsequent PCR steps, allowing researchers to use primers that are complementary to the adapter and the restriction site.

**160.** (D) AFLP markers are exclusively co-dominant in all plant species.
Molecular markers vary in their properties and applications. While RFLP requires Southern blotting, RAPD and AFLP are PCR-based. RAPD markers are usually dominant. It is incorrect to claim that AFLP markers are exclusively co-dominant; while they can sometimes show co-dominance, they are frequently analyzed as dominant markers because it is often difficult to distinguish heterozygotes based on band intensity alone.

**161.** (B) Point mutations that create or destroy a restriction enzyme recognition site
The generation of a Restriction Fragment Length Polymorphism is directly caused by changes in the DNA sequence. Point mutations, insertions, or deletions can either create a new recognition site for a restriction enzyme or destroy an existing one. When the DNA is digested, these changes result in fragments of different lengths, which are then visualized to detect genetic variation.

**162.** (B) Variable Number of Tandem Repeats (VNTRs)
Minisatellites are regions of the genome characterized by tandem repeats of DNA sequences, typically ranging from 10 to 100 base pairs in length. In the context of genetic analysis and DNA fingerprinting, these are technically referred to as Variable Number of Tandem Repeats. The number of repeats at these loci varies significantly between individuals, making them useful for personal identification.

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**127.** (B) A specific labeled single-stranded DNA or RNA sequence
In Northern blotting, the detection of specific RNA molecules is achieved using a labeled probe. This probe consists of a single-stranded DNA or RNA sequence that is exactly complementary to the target transcript. By hybridizing with the RNA on the membrane, the labeled probe allows researchers to visualize the location and relative abundance of the specific gene's expression.

**128.** (C) Dideoxynucleotides (ddNTPs) lack a 3'-hydroxyl group.
The Sanger sequencing method relies on the use of modified nucleotides called dideoxynucleotides. The critical chemical distinction between these and standard deoxyribonucleotides is that dideoxynucleotides lack a hydroxyl group at the 3' carbon of the sugar ring. This absence is fundamental to the technique, as it prevents the addition of further nucleotides during DNA synthesis, leading to chain termination.

**129.** (A) Missing 3'-OH prevents further phosphodiester bond formation.
DNA synthesis requires the formation of a phosphodiester bond between the 3'-hydroxyl group of the growing strand and the 5'-phosphate of the incoming nucleotide. When a dideoxynucleotide is incorporated, the lack of a 3'-hydroxyl group makes it impossible for the DNA polymerase to add the next nucleotide. This results in the immediate termination of the DNA chain at that position.

**130.** (B) Base-specific chemical modification and subsequent cleavage of the DNA backbone
The Maxam-Gilbert method is a chemical approach to DNA sequencing that does not rely on enzymatic synthesis. Instead, it is based on the principle of base-specific chemical modification followed by the subsequent cleavage of the DNA backbone at the modified sites. This process generates a set of labeled fragments of varying lengths, which are then separated by size to determine the sequence.

**131.** (B) Dimethyl sulfate (DMS)
In the original Maxam-Gilbert sequencing protocol, different chemicals are used to modify specific nitrogenous bases. To target guanine residues, the DNA is treated with dimethyl sulfate, which methylates the G bases. After modification, the DNA backbone is cleaved at these specific positions using piperidine. This reaction generates a pattern of fragments that identifies the location of guanines within the DNA sequence.

**132.** (D) Sequence is read via measuring pyrophosphate luminescence.
Automated Sanger sequencing has revolutionized genomics by utilizing fluorescently labeled dideoxynucleotides. This allows all four reactions to be combined in a single tube and separated in a capillary, with the results read as emission peaks on an electropherogram. It is incorrect to claim that the sequence is read via pyrophosphate luminescence; that mechanism is specific to pyrosequencing, not the Sanger method.

**133.** (A) Requirement to generate fragments terminating at every position.
The efficiency of Sanger sequencing depends on the precise balance between standard deoxynucleotides and chain-terminating dideoxynucleotides. The ratio is carefully chosen to ensure that the DNA polymerase incorporates a ddNTP only occasionally. This stochastic process generates a population of DNA fragments of every possible length, each terminating at a specific base, which is necessary for reading the full sequence.

**134.** (C) $T_7$ DNA polymerase
For optimal performance in Sanger sequencing, enzymes like $T_7$ DNA polymerase were modified to create a version known as Sequenase. A key modification was the removal of the 3' to 5' exonuclease activity, which normally serves as a proofreading mechanism. By eliminating this activity, the enzyme can no longer remove incorporated dideoxynucleotides, resulting in uniform bands and higher quality sequencing data.

**135.** (B) II, I, III, IV
The Maxam-Gilbert sequencing procedure follows a specific series of chemical and physical steps. For the guanine-specific reaction, the DNA is first treated with dimethyl sulfate to modify the bases. This is followed by treatment with piperidine to cleave the backbone at the modified sites. The resulting fragments are then separated on a high-resolution gel and visualized using autoradiography to read the sequence.

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**172.** (D) The melting temperature ($T_m$) of the primers
The annealing temperature in a PCR protocol is a critical parameter that must be optimized for specificity. It is typically set based on the melting temperature ($T_m$) of the primer pair used in the reaction. Usually, the annealing temperature is chosen to be a few degrees below the $T_m$ to ensure that the primers bind efficiently and specifically to their target.

**173.** (B) Significantly increases specificity and sensitivity of amplification.
Nested PCR is a modification designed to improve the performance of DNA amplification. Its primary advantage is that it significantly increases both the specificity and the sensitivity of the reaction. By using two successive rounds of PCR with two different sets of primers, the second set targeting a region within the first amplicon, any non-specific products from the first round are eliminated.

**174.** (B) Multiple distinct primer pairs in a single reaction tube.
Multiplex PCR is a technique that allows for the simultaneous amplification of several different DNA targets in a single reaction tube. This is achieved by including multiple distinct primer pairs, each specific to a different sequence. By carefully designing the primers to have similar melting temperatures and producing amplicons of different sizes, researchers can analyze many loci at once.

**175.** (A) IV, II, I, III
The process of RT-PCR begins with the annealing of primers, such as oligo(dT) or random primers, to the RNA template. Next, reverse transcriptase synthesizes the first-strand cDNA. Following synthesis, RNase H is used to degrade the original RNA template within the hybrid molecule. Finally, a thermostable DNA polymerase like Taq amplifies the specific cDNA target through standard PCR cycling.

**176.** (B) Generation of incomplete, truncated PCR products.
The extension time in a PCR cycle must be long enough for the DNA polymerase to complete the synthesis of the entire target sequence. If this time is set too short relative to the size of the expected amplicon, the enzyme will fall off the template before reaching the end. This results in the generation of incomplete, truncated PCR products rather than full-length pieces.

**177.** (C) It possesses a robust 3' to 5' proofreading exonuclease activity.
Taq DNA polymerase is a thermophilic enzyme that is central to the PCR technique, functioning optimally at 72 degrees Celsius. It is known for its ability to add a single adenine overhang to the 3' end of its products. However, it is incorrect to state that it possesses robust 3' to 5' proofreading activity. Its lack of this exonuclease activity results in a higher error rate.

**178.** (B) It acts as a necessary cofactor for the catalytic activity of DNA polymerase.
Magnesium ions are an essential component of the PCR reaction mixture. They function as a necessary cofactor for the catalytic activity of the DNA polymerase enzyme. The $Mg^{2+}$ ions coordinate with the phosphate groups of the dNTPs and the DNA template, facilitating the formation of the phosphodiester bond. Proper magnesium concentration is critical for both the yield and specificity of the amplification.

**179.** (A) The minor groove of double-stranded DNA
SYBR Green I is a fluorescent dye widely used in real-time PCR for the quantification of DNA. It functions by binding specifically to the minor groove of double-stranded DNA molecules. When the dye is free in solution, it exhibits very little fluorescence, but upon binding to the accumulated PCR products, its fluorescence intensity increases dramatically, allowing for the real-time monitoring of amplification.

**180.** (B) Probe cleavage by Taq exonuclease releases the fluorophore.
A TaqMan probe is a specialized oligonucleotide used in qPCR that contains a fluorescent reporter and a quencher. During the extension phase, the Taq polymerase encounters the probe hybridized to the target sequence. The enzyme's intrinsic 5' to 3' exonuclease activity then cleaves the probe, separating the fluorophore from the quencher. This release allows the fluorophore to emit a measurable signal.

**181.** (B) The cycle at which the fluorescence signal crosses a defined background threshold, marking the exponential phase
The Ct value, also known as the threshold cycle or Cq, is the most important measurement in a

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**163.** (A) SSR repeat units are shorter (typically 1-6 bp) compared to VNTR repeat units (10-100 bp).
Microsatellites, also known as Simple Sequence Repeats, differ from minisatellites primarily in the length of their repeat units. SSRs typically consist of very short motifs, usually one to six base pairs, whereas minisatellites have longer repeat units of ten to one hundred base pairs. Both types of tandem repeats exhibit high levels of length polymorphism and are widely used in genetics.

**164.** (B) DNA polymerase slippage during replication
The high level of polymorphism found at microsatellite loci is primarily attributed to a process called DNA polymerase slippage. During DNA replication, the polymerase can lose its place on the repetitive template, leading to the insertion or deletion of one or more repeat units in the new strand. This frequent error results in the variation in repeat number observed within populations.

**165.** (B) P-1, Q-2, R-3, S-4
Molecular markers utilize different genetic principles for analysis. SNPs are based on single base pair variations. SSRs measure length variation in short tandem repeats. RFLPs involve changes in the length of restriction fragments detected through hybridization. AFLPs rely on the PCR amplification of specific restriction fragments using adapter-directed primers. Each method provides unique insights into the genetic makeup of an organism.

**166.** (C) PCR multiplexing of highly polymorphic STR (microsatellite) loci
Modern forensic DNA profiling relies heavily on the analysis of Short Tandem Repeats, which are a type of microsatellite. This method utilizes PCR to simultaneously amplify multiple highly polymorphic STR loci. Because these regions are very small and can be multiplexed, the technique is extremely sensitive, capable of generating a unique genetic profile from very tiny or degraded biological samples.

**167.** (B) They are typically multi-allelic, with up to four possible alleles per locus common in populations.
Single Nucleotide Polymorphisms are the most common form of genetic variation in humans, occurring in both coding and non-coding DNA. They are highly useful for genomic studies and can be detected with microarrays. However, it is incorrect to state that they are typically multi-allelic. Most SNPs are bi-allelic, meaning there are only two possible versions of the sequence at that locus.

**168.** (B) A mixture of common alleles for specific STR loci used as a size reference standard
An allelic ladder is a specialized reference standard used in forensic DNA analysis when genotyping STR loci. It consists of a mixture of the most common alleles found in the population for each specific locus being tested. By comparing the unknown samples to the ladder during electrophoresis, researchers can accurately identify the number of repeats and assign the correct genotype.

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**169.** (B) An allele that fails to amplify during PCR due to a mutation in the primer binding site
In the context of microsatellite genotyping, a null allele occurs when a specific allele fails to be amplified during a PCR reaction. This lack of amplification is typically caused by a mutation, such as a SNP or a small indel, within the sequence where the PCR primer is supposed to bind. As a result, the individual may be incorrectly identified as homozygous.

**170.** (C) RNA primase
A standard Polymerase Chain Reaction requires several essential components, including a thermostable DNA polymerase, deoxynucleotide triphosphates, and a pair of sequence-specific DNA primers. However, RNA primase is not needed for the reaction. In PCR, the necessary primers are provided as synthetic DNA oligonucleotides that anneal directly to the template, fulfilling the requirement for a free 3'-hydroxyl group.

**171.** (B) The double-stranded DNA template denatures into single strands.
During the thermal cycling of a PCR reaction, the step performed at 94 to 95 degrees Celsius is the denaturation phase. At this high temperature, the hydrogen bonds between the complementary bases of the double-stranded DNA template are broken. This causes the DNA to separate into two individual single strands, which then serve as templates for the subsequent annealing and extension steps.

experimental tissues, are labeled with distinct fluorescent dyes, Cy3 and Cy5. These two samples are then hybridized competitively to the same microarray. The ratio of the green and red fluorescence at each spot represents the relative abundance of that specific transcript in the two compared samples.

**191.** (A) Removes technical variation and systematic biases from data.
Data normalization is a crucial computational step in microarray analysis that aims to remove technical variation and systematic biases. These biases can arise from differences in labeling efficiency, scanning settings, or physical variations across the array. By applying normalization methods like Lowess, researchers ensure that the observed differences in signal intensity truly reflect biological changes rather than artifacts of the experimental process.

**192.** (B) They are strictly limited to the detection of nucleic acid sequences.
Protein microarrays are powerful tools used for the large-scale analysis of protein functions and interactions. They utilize capture molecules such as antibodies or recombinant proteins to study activities like kinase phosphorylation. It is incorrect to state that they are strictly limited to the detection of nucleic acid sequences, as their primary purpose is to profile the proteome and investigate various protein-related biological questions.

**193.** (B) Detecting chromosomal copy number deletions and duplications.
Comparative Genomic Hybridization using microarrays is a specialized technique designed to detect chromosomal imbalances. By hybridizing genomic DNA from a test sample and a reference sample to the array, researchers can identify copy number variations, such as deletions or duplications, across the entire genome. This method is widely used in clinical diagnostics to detect genetic causes of developmental delays and cancer.

**194.** (B) RNA is highly unstable and prone to degradation during the protocol.
In microarray experiments, target RNA is typically converted into cDNA or cRNA before labeling. This step is necessary because RNA is a highly unstable molecule that is prone to rapid degradation by ubiquitous RNase enzymes during the long hybridization and washing procedures. By creating more stable DNA or complementary RNA copies, researchers can ensure the integrity of the genetic information.

**195.** (B) Absorbs laser energy to facilitate soft analyte ionization.
In Matrix-Assisted Laser Desorption/Ionization, the matrix molecule plays a vital role in the ionization process. It serves to absorb the energy from the laser pulse, which protects the larger analyte molecules from being destroyed. The matrix then facilitates the soft ionization and sublimation of the proteins or peptides into the gas phase, allowing them to be analyzed by the mass spectrometer.

**196.** (B) ESI generates multiply charged liquid ions; MALDI generates solid singly charged ions.
ESI and MALDI are the two primary ionization techniques used in mass spectrometry. They differ in their physical state and the ions they produce. ESI is performed in the liquid phase and typically generates multiply charged ions, which is ideal for coupling with chromatography. MALDI involves a solid-phase matrix and usually produces singly charged ions, often being paired with time-oF-flight analyzers.

**197.** (A) Sequence-specific cleavage of the purified protein into peptides using an enzyme like Trypsin
The initial and most critical step in Peptide Mass Fingerprinting is the sequence-specific cleavage of the purified protein. This is typically achieved using a protease such as Trypsin, which digests the protein into a predictable set of smaller peptides. The masses of these resulting peptides are then measured by mass spectrometry to create a "fingerprint" that can be used for protein identification.

**198.** (A) By recording the time it takes for an accelerated ion to travel a fixed distance through a field-free drift tube
A Time-oF-Flight mass analyzer determines the mass-to-charge ratio of an ion by measuring how long it takes to travel through a vacuum tube. After being accelerated by an electric field, all ions have the same kinetic energy. Lighter ions travel faster and reach the detector sooner than heavier ions. This precise timing allows the system to calculate the exact mass of each ion.

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real-time PCR assay. It is defined as the cycle number at which the fluorescence signal first rises above the background noise and crosses a predefined threshold. This point marks the exponential phase of the reaction, and the value is inversely proportional to the initial target concentration.

**182.** (A) Both A and R are true and R is the correct explanation of A.
In SYBR Green-based qPCR, a post-amplification melt curve analysis is essential for verifying the results. Because the dye binds non-specifically to all double-stranded DNA, the measured fluorescence can include signals from primer dimers and ofF target amplicons as well as the desired product. The melt curve allows researchers to distinguish between these different DNA species based on their unique melting temperatures.

**183.** (C) Conformational Molecular Beacons.
Molecular Beacons are a type of qPCR probe that utilizes a stem-and-loop structure to control fluorescence. The loop contains the target-specific sequence, while the stem keeps the fluorophore and quencher in close proximity. When the probe hybridizes to its target, it undergoes a conformational change that separates the two moieties, generating a signal without the need for the probe to be cleaved.

**184.** (A) Two adjacent probes hybridize to the target, bringing a donor and acceptor fluorophore into close proximity to enable energy transfer.
In FRET-based qPCR assays, such as those used in the LightCycler system, the signal is generated by two separate probes. These probes are designed to hybridize to adjacent sites on the target DNA. When both probes are bound, the donor fluorophore on one probe and the acceptor on the other are brought into close proximity, enabling the non-radiative transfer of energy between them.

**185.** (C) I, II and III
Real-time PCR quantification involves several key principles. A lower Ct value indicates a higher starting concentration of the target. Absolute quantification requires comparing samples to a standard curve of known values, while relative quantification involves normalizing the data to a stable reference gene. It is incorrect to say that amplification efficiency has no effect; it is actually a major factor in Ct determination.

**186.** (B) They are consumed and degraded during the extension phase of qPCR.
Molecular Beacons are highly specific probes that utilize a stem-loop structure to distinguish even single nucleotide differences. The loop part of the probe is complementary to the target DNA. It is incorrect to state that they are consumed and degraded during the extension phase. Unlike TaqMan probes, Molecular Beacons can re-fold and be reused in each cycle of the PCR reaction.

**187.** (A) Standard measures plateau phase; qPCR measures exponential phase.
The fundamental difference between standard PCR and quantitative real-time PCR lies in how the DNA is measured. Standard PCR is an end-point assay where the products are analyzed during the plateau phase, often using gel electrophoresis. In contrast, qPCR monitors the accumulation of DNA in real time during the exponential phase, allowing for the precise quantification of the starting material.

**188.** (B) The known DNA sequence immobilized onto the solid surface
In the standardized terminology of microarray technology, the term "probe" refers to the known DNA sequence that is immobilized onto the solid surface of the chip. These probes are designed to be complementary to specific target sequences in the sample. The unknown fluorescently labeled nucleic acid molecules that are applied to the array in solution are referred to as the "targets."

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**189.** (B) Photolithography utilizing light-directed chemical synthesis
High-density oligonucleotide microarrays, such as Affymetrix GeneChips, are produced using an in situ fabrication process. This method utilizes photolithography, which involves light-directed chemical synthesis on the chip surface. By using specific masks and light-sensitive protecting groups, researchers can build tens of thousands of unique oligonucleotide probes directly on the glass substrate, one nucleotide at a time, with very high precision.

**190.** (A) Two distinct samples hybridized competitively to the array.
In a two-color cDNA microarray experiment, two different samples, such as control and

**208.** (B) Distinct, non-overlapping epitopes on the target analyte.
In a sandwich ELISA configuration, the target analyte is captured and detected using two different antibodies. To function correctly, these two antibodies must recognize and bind to distinct, non-overlapping epitopes on the same analyte molecule. This "sandwich" arrangement ensures high specificity, as the signal is only generated when both antibodies successfully bind to the target at the same time.

**209.** (A) Microspheres dyed with unique fluorophore ratios for capture.
The Luminex bead-based assay is a multiplex technology that uses color-coded microspheres for detection. Each set of beads is dyed with a unique ratio of two different fluorophores, creating a specific spectral signature. These beads are then coated with capture probes for different analytes. This allows the system to identify which analyte is being measured based on the bead's color during analysis.

**210.** (A) One identifies the analyte bead; the other quantifies binding.
In a Luminex flow cytometry system, two distinct lasers are used to analyze each bead as it passes through the detection chamber. The first laser, often a red laser, excites the internal dyes of the microsphere to identify which specific analyte set the bead belongs to. The second laser, usually green, excites the reporter molecule to quantify the amount of analyte binding.

**211.** (B) Colored nanoparticles conjugated to the detection antibody.
Visual detection in a lateral flow assay is achieved through the use of colored nanoparticles, such as colloidal gold or colored latex beads. These particles are conjugated to a detection antibody that is specific for the target analyte. When the target is present, these labeled complexes accumulate at the test line, creating a visible colored band that can be seen without equipment.

**212.** (C) Both the test line and the control line
In a standard lateral flow immunochromatographic assay, a positive result is indicated by the appearance of two distinct colored lines. The first is the test line, which captures the target-analyte complex. The second is the control line, which captures excess detection reagents. Both lines must be colored for the test to be considered both positive and valid, ensuring the reagents worked.

**213.** (A) Confirms wicking through the strip and reagent activity.
The control line in a lateral flow assay serves a vital role in quality control. Its appearance confirms that the liquid sample has successfully wicked through the entire length of the strip and that the labeled reagents are biologically active. If the control line fails to appear, the test is considered invalid regardless of the result at the test line, indicating a failure.

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**214.** (D) They generally require highly trained personnel and extensive sample preparation.
Point-of-care rapid technologies are designed for speed and convenience, providing actionable results near the patient. Formats like lateral flow tests and microfluidic paper devices are specifically developed to be simple to use. It is incorrect to state that they require highly trained personnel or extensive sample preparation, as their goal is to be accessible to users in various non-laboratory settings.

**215.** (D) P-3, Q-4, R-2, S-1
Solid-phase peptide synthesis involves several key chemical components. The Merrifield resin serves as the insoluble polymer support. The Fmoc group acts as a temporary protecting group for the alpha-amino position. Reagents like DCC or HATU are used to activate the carboxyl group for coupling. Finally, a mild base such as piperidine is used to remove the Fmoc group during each cycle.

**216.** (B) From the C-terminus to the N-terminus
In solid-phase peptide synthesis, the assembly of the polypeptide chain occurs in a direction that is the opposite of natural ribosomal translation. The process begins with the C-terminal amino acid being attached to the insoluble resin support. Subsequent amino acids are then added one by one to the N-terminus of the growing chain, meaning the synthesis proceeds from the C-terminus to the N-terminus.

**217.** (A) Requires corrosive TFA for deprotection and lethal HF for cleavage.
The Boc-strategy for peptide synthesis is considered more demanding and hazardous than the modern Fmoc-strategy due to the reagents required. It relies on the use of

corrosive trifluoroacetic acid for the repeated deprotection of the amino groups during each cycle. Furthermore, the final cleavage of the peptide from the resin requires highly lethal and extremely hazardous anhydrous hydrogen fluoride, which requires specialized equipment.

**218.** (A) Excess reagents are easily removed by filtration and washing.
The use of an insoluble resin support, a concept pioneered by Bruce Merrifield, revolutionized peptide synthesis. The fundamental advantage is that it allows the growing peptide to remain fixed to a solid surface throughout the process. This enables excess reagents and soluble byproducts to be easily removed after each step simply by filtering and washing the resin, leading to much higher yields and purity.

**219.** (D) They cannot mimic the post-translational modifications of native proteins.
Synthetic peptides are versatile tools used as antigens for antibody production and as lead compounds for drug development. Solid-phase synthesis allows for the incorporation of unnatural amino acids, such as D-isomers. It is incorrect to claim they cannot mimic post-translational modifications; in fact, techniques in SPPS can be used to add modifications like phosphorylation or glycosylation to the synthetic chain.

**220.** (D) Concentrated Trifluoroacetic acid (TFA) with scavengers
In the Fmoc strategy of solid-phase peptide synthesis, the final step involves the removal of the peptide from the resin and the clearing of side-chain protecting groups. This is typically accomplished using a solution of concentrated trifluoroacetic acid. Scavengers are usually added to this TFA mixture to prevent the reactive chemical species generated during the process from damaging the sensitive amino acid residues.

**221.** (A) Accumulated incomplete couplings reduce final full-length yield.
Synthesizing long peptides exceeding 50 amino acids presents a significant challenge due to the accumulation of incomplete coupling reactions. Even if each step is highly efficient, the small percentage of failed reactions adds up over many cycles. This leads to a decrease in the final yield of the full-length peptide and complicates the purification process because of the presence of many truncated sequences.

**222.** (B) Water or ethanedithiol (EDT)
During the final cleavage of a peptide using trifluoroacetic acid, highly reactive carbocations are released as protecting groups are removed. To prevent these cations from undergoing undesirable side reactions with the peptide residues, scavenger molecules are added to the mixture. Common scavengers include water, triisopropylsilane, or ethanedithiol, which react with and neutralize these harmful species, protecting the integrity of the final product.

**223.** (A) To enhance its resistance to proteolytic degradation in vivo
For therapeutic applications, synthetic peptides are sometimes designed using D-amino acids instead of the natural L-isomers. The primary purpose of this substitution is to enhance the peptide's resistance to proteolytic degradation within the body. Because most natural enzymes only recognize L-amino acids, the use of D-amino acids significantly increases the biological halF-life and stability of the drug in the bloodstream.

**224.** (A) I, III, IV, II
A single cycle of amino acid addition in Fmoc solid-phase peptide synthesis follows a logical order. First, the Fmoc protecting group is removed from the resin-bound amino acid, followed by washing. Next, the incoming Fmoc-amino acid is activated using a coupling reagent. Finally, this activated amino acid is coupled to the free amino group of the resin-bound molecule, and the resin is washed.

**225.** (A) I, II, III, V
Peptide synthesis involves several key differences from biological translation. While SPPS proceeds in the C-to-N direction, ribosomal translation occurs from the N-to-C terminus. In chemical synthesis, the Fmoc group is removed using a mild base, whereas the Boc group requires acid. Protecting groups on the amino acid side chains are essential to prevent branching and ensure the formation of a linear peptide.

**226.** (B) It denatures proteins, causing them to partition into the organic phase or at the interphase.
During the isolation of nucleic acids, phenol is used as a powerful solvent for protein

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denaturation. When added to a cellular lysate, phenol disrupts the structures of proteins, causing them to lose their solubility in water. As a result, the denatured proteins partition into the lower organic phase or accumulate at the interphase, while the hydrophilic DNA and RNA remain in the aqueous phase.

**227.** (A) To prevent foaming and stabilize the phase boundary
Isoamyl alcohol is a common additive in phenol-chloroform extractions. Its primary function is to act as an anti-foaming agent, which helps to stabilize the boundary between the aqueous and organic phases. By preventing the formation of persistent foam during the mixing process, it makes the subsequent separation and recovery of the nucleic acid-containing aqueous layer easier and more efficient.

**228.** (B) Isopropanol or Ethanol
Nucleic acids are routinely recovered from aqueous solutions through the process of alcohol precipitation. In the presence of monovalent cations, the addition of either isopropanol or ethanol serves to lower the dielectric constant of the solution. This allows the positive ions to neutralize the negative charges on the DNA or RNA backbone, causing the molecules to become insoluble and precipitate out of solution.

**229.** (D) They specifically degrade genomic DNA without affecting RNA.
Chaotropic salts, such as guanidinium thiocyanate, are essential for successful RNA isolation. They function as powerful protein denaturants that effectively inhibit the activity of intrinsic RNases, which would otherwise destroy the RNA. They also promote the binding of RNA to silica membranes. It is incorrect to claim they specifically degrade genomic DNA; rather, they serve to protect and facilitate the recovery of RNA.

**230.** (A) Hardens the lipid bilayer to stabilize the competent state.
When preparing competent E. coli cells using methods like the rubidium chloride technique, it is vital to keep the cells continuously on ice. The low temperature helps to harden the lipid bilayer of the cell membrane, which stabilizes the fragile competent state. This cooling prevents the premature resealing of the temporary openings or pores, ensuring that the cells remain ready for DNA uptake.

**231.** (B) It cleaves at a precise, defined distance outside of its asymmetric recognition sequence.
Type IIS restriction endonucleases are a specialized class of enzymes that differ from the more common Type II variants. Their defining feature is that they recognize an asymmetric DNA sequence and cleave the double helix at a precise and defined distance outside of that recognition site. This property makes them highly valuable for advanced cloning techniques where specific overhangs are required.

**232.** (A) Removes recognition sites for scarless, seamless assembly.
The primary advantage of using Type IIS restriction enzymes in techniques like Golden Gate assembly is the ability to perform scarless cloning. Because these enzymes cut outside of their recognition sequence, the original site can be removed from the final product during the ligation process. This allows multiple DNA fragments to be joined seamlessly without leaving behind any unwanted restriction site sequences.

**233.** (B) In-frame insertion that altered but did not abolish activity.
The appearance of a pale blue colony in blue-white screening suggests that some beta-galactosidase activity is still present. This most likely occurs because of an in-frame insertion of foreign DNA that altered the lacZ-alpha sequence but did not completely abolish its function. The resulting fusion protein retains enough enzymatic capability to partially cleave X-gal, leading to a faint rather than a dark blue color.

**234.** (B) I, IV, II, III
The construction of a genomic library follows a specific sequence of molecular biology steps. First, the genomic DNA is partially digested with restriction enzymes to produce large overlapping fragments. These fragments are then size-fractionated to select the optimal length for cloning. Next, the DNA is ligated into a suitable vector, such as a lambda phage, and finally packaged into phage heads for delivery.

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**235.** (B) Generates overlapping fragments for chromosome walking and mapping.
Partial digestion is a critical technique when constructing a representative genomic library.

Unlike complete digestion, which cuts at every recognition site, partial digestion leaves some sites untouched. This generates a population of large, overlapping DNA fragments. These overlaps are essential for downstream applications like chromosome walking and genomic mapping, as they allow researchers to reassemble the original sequence of the genome.

**236.** (A) cDNA inserted near a promoter for transcription/translation.
An expression library is a specialized collection of clones designed to produce the protein product of the inserted genes. In contrast to a standard genomic library, it uses cDNA synthesized from mRNA, which is then inserted downstream of a functional promoter in the vector. This allows the host cell to transcribe and translate the eukaryotic gene into a functional protein for screening.

**237.** (C) Colony hybridization
Colony hybridization is a technique used to screen for specific DNA sequences within a large population of bacterial clones. The process involves transferring the DNA directly from bacterial colonies growing on an agar plate onto a nitrocellulose membrane. The cells on the membrane are then lysed and the DNA is denatured, allowing for the subsequent hybridization with a labeled probe to identify recombinants.

**238.** (B) Lysis of the host bacterial cells by multiplying recombinant phages
In a plaque hybridization assay used to screen a bacteriophage library, the results are visualized on a lawn of bacterial cells. The clear zones, known as plaques, are created by the iterative cycle of infection and lysis of the host bacteria by multiplying recombinant phages. Each plaque represents a clonal population originating from a single phage, making it easier to isolate the target.

**239.** (B) Contains introns and extensive genomic regulatory regions.
A cDNA library is a valuable resource for studying gene expression as it represents the actively transcribed genes of a specific tissue. It is synthesized from mRNA using reverse transcriptase and simplifies cloning into prokaryotic systems. It is incorrect to state that it contains introns and extensive genomic regulatory regions, as these are typically removed during the natural processing of mRNA in eukaryotes.

**240.** (A) Inclusion of oligonucleotide linkers with specific restriction sites.
To facilitate the efficient insertion of cDNA molecules into a cloning vector, researchers frequently modify the 5' ends. This is commonly done by ligating synthetic oligonucleotide linkers that contain specific restriction enzyme recognition sites. After ligation, these linkers are digested to create cohesive ends that are perfectly compatible with the prepared vector, ensuring a higher rate of successful recombinant formation.

**241.** (A) Nicks hybrid RNA for 3'-OH second-strand DNA primers.
During the synthesis of double-stranded cDNA, the enzyme RNase H plays a critical role. After the first DNA strand is synthesized from the RNA template, RNase H is used to introduce nicks and gaps into the hybrid RNA strand. These resulting RNA fragments then serve as essential primers for the DNA polymerase to synthesize the second strand of the cDNA molecule.

**242.** (C) To map the specific in vivo binding sites of proteins (e.g., transcription factors) on DNA
Chromatin Immunoprecipitation is a powerful technique used to study the interactions between proteins and DNA within the cell nucleus. Its primary purpose is to map the specific in vivo binding sites of various proteins, such as transcription factors or histones, on the genome. By capturing these complexes, researchers can determine which genes are being regulated by specific proteins under various cellular conditions.

**243.** (B) Formaldehyde
In a standard ChIP assay, the first step involves stabilizing the protein-DNA interactions that occur naturally in living cells. This is typically achieved by treating the cells with formaldehyde, which acts as a reversible crosslinking agent. Formaldehyde creates covalent bonds between proteins and DNA that are in close proximity, allowing the complexes to remain intact throughout the subsequent lysis and purification steps.

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**244.** (C) Complete target DNA digestion by exonucleases before sequencing.
The ChIP-seq workflow involves crosslinking, chromatin shearing by sonication or enzymes, and immunoprecipitation with specific antibodies. It is incorrect to include a step where the target DNA is completely digested by exonucleases before sequencing. In fact, the DNA must remain intact enough to be sequenced and mapped back to the genome to identify the precise binding locations of the target protein.

**245.** (A) Serial Analysis of Gene Expression (SAGE)
Serial Analysis of Gene Expression is a method used to evaluate global gene expression patterns. It works by generating and counting short sequence tags, typically 10 to 14 base pairs long, from the 3' ends of all transcripts in a sample. By recording the frequency of each unique tag, researchers can quantify the relative expression levels of thousands of different genes simultaneously.

**246.** (B) Detects novel transcripts and splice variants without prior knowledge.
RNA-seq differs fundamentally from DNA microarrays because it uses high-throughput sequencing rather than hybridization to pre-defined probes. This allows RNA-seq to detect novel transcripts, alternative splice variants, and gene fusions without any prior knowledge of the genome. Additionally, it offers a wider dynamic range and higher sensitivity for quantifying expression levels, making it the current standard for transcriptome profiling.

**247.** (A) A DNA-protein complex migrates more slowly through a non-denaturing gel than free DNA.
The Electrophoretic Mobility Shift Assay is a technique used to study DNA-protein interactions. It is based on the fundamental principle that a DNA-protein complex has a larger molecular mass and different charge than free DNA. Consequently, when run on a non-denaturing gel, the complex will migrate more slowly than the unbound DNA probe, resulting in a characteristic "shift" in the banding pattern.

**248.** (A) A "supershift" generated by adding a specific antibody directed against the target protein
In an EMSA experiment, a "supershift" is used to confirm the identity of the specific protein bound to the DNA. This is achieved by adding a specific antibody directed against the target protein to the reaction mixture. If the protein is present in the complex, the antibody will bind to it, creating an even larger complex that migrates even more slowly in the gel.

**249.** (D) DNase I Footprinting
DNase I Footprinting is the most appropriate method for identifying the exact nucleotides within a DNA sequence where a transcription factor binds. Unlike techniques that only show that binding has occurred, footprinting reveals the precise boundary of the protein-DNA interface. This information is crucial for understanding the structural basis of gene regulation and the specific DNA motifs recognized by different proteins.

**250.** (B) Exposes DNA to excessive DNase I to degrade all regions.
In DNase I Footprinting, one strand of DNA is end-labeled and then exposed to the protein of interest. It is incorrect to say the DNA is exposed to excessive DNase I; rather, a limited amount of the enzyme is used to ensure only one cleavage per molecule on average. This creates a ladder of fragments where the protein-bound region is protected from digestion.

**251.** (A) A blank area (missing bands) in the cleavage ladder corresponding to the protein-bound protected sequence
On the final autoradiograph of a footprinting assay, the "footprint" appears as a blank area or a series of missing bands in the cleavage ladder. This gap corresponds directly to the specific DNA sequence that was protected by the bound protein. By comparing this pattern to a control lane without protein, researchers can identify the exact nucleotides involved in the interaction.

**252.** (D) Luciferase
Luciferase is a popular reporter gene that produces a bioluminescent signal. Unlike Green Fluorescent Protein, which requires an external light source to excite the fluorophore, luciferase generates light through a chemical reaction. By oxidizing its substrate, luciferin, the enzyme emits photons that can be detected and quantified, providing a highly sensitive measurement of gene expression with very low background noise.

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elastomer that is easy to mold into complex channel patterns. Its transparency allows for easy microscopic observation of fluid flow, while its gas permeability is beneficial for cell culture applications. Its low cost and biocompatibility make it a standard choice in microfluidic research.

**289.** (B) Electro-osmotic flow generated by an applied electric field
In many microfluidic applications, fluid movement is controlled using electro-osmotic flow rather than mechanical pumps. This is achieved by applying an electric field across the channels. The field interacts with the electric double layer that naturally forms at the channel surface, causing the entire liquid column to move uniformly. This method allows for precise control of fluid flow and sample separation.

**290.** (B) Encapsulating cells in partitioned oil droplets for reactions.
Droplet-based microfluidics is a powerful technique used for high-throughput analysis, such as single-cell RNA sequencing. The method works by encapsulating individual cells into separate oil droplets, which act as partitioned reaction chambers. Inside each droplet, the cell is lysed and its contents are processed, allowing researchers to analyze thousands of single cells simultaneously while maintaining the link between the cell and its data.

**291.** (D) PDMS absorbs small hydrophobic molecules, altering concentrations.
A significant limitation of using PDMS in microfluidic devices is its tendency to absorb small hydrophobic molecules. This can lead to inaccurate results in drug testing or biochemical assays, as the concentration of the target molecules in the fluid is reduced as they seep into the porous polymer matrix. Researchers must often treat the surface or use alternative materials to avoid this problem.

**292.** (A) Sample pumping through fixed temperature zones on the chip.
In a continuous-flow microfluidic PCR device, thermal cycling is achieved by moving the sample through different temperature zones on the chip. Instead of heating and cooling a single chamber, the fluid is pumped through a channel that passes over fixed heaters set to denaturation, annealing, and extension temperatures. This spatial cycling allows for rapid and continuous amplification of DNA as the fluid flows.

**293.** (B) Replicating functional organ units in vitro for physiology modeling.
Organ-on-a-chip technology is an emerging field that uses microfluidics to replicate the functional units of human organs. By culturing living cells in a controlled microenvironment that mimics physiological conditions, such as mechanical strain and fluid flow, researchers can model organ function and disease. This provides a more accurate and efficient platform for drug screening and toxicity testing than traditional cell cultures.

**294.** (D) It requires a standard curve for absolute quantification.
Digital PCR is an advanced method for absolute quantification that partitions a sample into thousands of tiny reactions. It uses Poisson statistics to calculate the initial number of target molecules based on the number of positive and negative partitions. It is incorrect to state that it requires a standard curve; in fact, its primary advantage is the ability to provide absolute measurements without one.

**295.** (B) Dilution such that a fraction of partitions receives zero targets.
A fundamental requirement for a successful digital PCR assay is the proper partitioning of the target molecules. The sample must be diluted so that some of the partitions contain the target DNA while others contain zero molecules. This distribution follows a Poisson pattern, which allows for the absolute concentration to be calculated by counting the number of positive and negative signals.

**296.** (C) By droplet flow through fluorescence detectors for amplitude.
In droplet digital PCR, the positive and negative partitions are distinguished by measuring their fluorescence intensity after the reaction has reached its end-point. The droplets are passed through a fluorescence detector in a flow-stream, which records the amplitude of the signal from each one. Droplets with a high signal are counted as positive for the target, while those with background levels are negative.

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gene inside, researchers can easily isolate and identify specific binders.

**262.** (B) A coat protein (e.g., pIII or pVIII of filamentous phage $M_{13}$)
In the construction of a phage display library, the gene encoding the protein of interest is typically fused to a gene for a phage coat protein. For filamentous phages like $M_{13}$, the most common choices are the minor coat protein pIII or the major coat protein pVIII. This fusion ensures that the recombinant protein is incorporated into the phage particle and displayed externally.

**263.** (B) Panning
Panning is the iterative selection process used in phage display to isolate specific binding molecules from a vast library. It involves incubating the library with a target, washing away non-binding phages, and then eluting the bound phages. These eluted phages are then amplified in bacteria and the cycle is repeated multiple times to enrich for the highest affinity binders for the target.

**264.** (D) Cleavage requires a PAM sequence located upstream of guide binding.
CRISPR-Cas9 is a revolutionary genome editing tool where Cas9 acts as an RNA-guided endonuclease to induce double-strand breaks. The sgRNA provides the specificity by binding to a 20-nucleotide target sequence. It is incorrect to say the PAM sequence is located upstream; in reality, the Protospacer Adjacent Motif must be located immediately downstream of the target sequence for cleavage to occur.

**265.** (B) It cleaves exactly 3 base pairs upstream of the PAM sequence.
The precise location of DNA cleavage by the Cas9 protein is strictly determined by the position of the PAM sequence. Once the Cas9-sgRNA complex binds to the target DNA, the enzyme induces a double-strand break at a specific point. This cleavage typically occurs exactly three base pairs upstream of the PAM, providing a highly predictable and programmable method for genome modification.

**266.** (A) Non-Homologous End Joining (NHEJ)
Following a double-strand break induced by Cas9, the cell attempts to repair the damage. The primary pathway utilized for generating gene knockouts is Non-Homologous End Joining. This repair mechanism is inherently error-prone and often introduces random insertions or deletions at the site of the break. These indels can lead to frameshift mutations, effectively disrupting the function of the target gene.

**267.** (A) A donor DNA template containing the desired mutation flanked by homologous sequences
To introduce a specific point mutation using CRISPR-Cas9, the researcher must utilize the Homology-Directed Repair pathway. This requires providing a donor DNA template along with the Cas9 and sgRNA. The donor DNA must contain the desired mutation and be flanked by sequences homologous to the region around the break. The cell's repair machinery then uses this template to accurately edit the genome.

NODIA

**268.** (D) Catalytically dead Cas9 (dCas9) fused to a transcriptional repressor
CRISPR interference is a method used to repress the expression of specific genes without altering the DNA sequence. This technique utilizes a catalytically dead version of the Cas9 protein, known as dCas9. By fusing dCas9 to a transcriptional repressor, the complex can be guided to a promoter or coding region, where it physically blocks the transcription machinery from accessing the DNA.

**269.** (B) RNA-guided base pairing avoids complex protein engineering.
A major advantage of the CRISPR-Cas system compared to earlier technologies like ZFNs and TALENs is its simplicity and ease of design. While ZFNs and TALENs require complex protein engineering to recognize every new DNA target, CRISPR relies on simple RNA-guided base pairing. This makes the system much faster and more cost-effective to implement, as researchers only need to synthesize a new sgRNA.

**270.** (B) It cleaves long double-stranded RNA (dsRNA) or short hairpin RNA (shRNA) into ~21-23 nucleotide siRNAs.
In the RNA interference pathway, the Dicer enzyme plays a central role in processing double-stranded RNA. It is an RNase III-type enzyme that recognizes and cleaves long dsRNA or short hairpin RNA molecules into smaller fragments. These resulting molecules, typically 21 to 23 nucleotides in length, are called small interfering RNAs and are essential for the subsequent silencing of target gene expression.

**253.** (A) It acts as an internal control for normalization of transfection efficiency and cell viability.
In a dual-luciferase reporter assay, two different enzymes are used to monitor gene activity. The experimental promoter drives the expression of firefly luciferase, while a second enzyme, typically Renilla luciferase, is expressed from a constitutive promoter. The Renilla signal serves as an internal control for normalization, correcting for variations in transfection efficiency, cell viability, and sample processing between different experimental wells.

**254.** (B) ATP-dependent oxidation of luciferin yielding photons.
The Firefly luciferase enzyme catalyzes a bioluminescent reaction that is both ATP-dependent and requires oxygen. The primary biochemical process involves the oxidation of the substrate beetle luciferin to oxyluciferin. During this conversion, energy is released in the form of visible light photons. The intensity of this emitted light is directly proportional to the amount of active luciferase enzyme present in the sample.

**255.** (D) It requires the two interacting proteins to be localized to the cell membrane.
The Yeast Two-Hybrid system is a powerful in vivo technique for detecting protein-protein interactions. It works by splitting a transcription factor into a DNA-binding domain and an activation domain, each fused to one of the interacting proteins. It is incorrect to claim the proteins must be localized to the membrane; in fact, the interaction must occur within the nucleus to activate reporter genes.

**256.** (A) The Bait
In the nomenclature of the Yeast Two-Hybrid system, the target protein that is fused to the DNA-Binding Domain is known as the "bait." The bait is the protein being studied to find its interacting partners. The partner protein, which is fused to the transcriptional activation domain, is referred to as the "prey," which is screened from a library to identify interactions.

**257.** (D) Bait protein has intrinsic activity and activates reporters alone.
A common cause of false positives in a Yeast Two-Hybrid assay is the phenomenon known as auto-activation. This occurs when the bait protein itself possesses an intrinsic ability to activate the reporter gene without needing to interact with a prey protein. Such bait proteins can lead to the misleading appearance of an interaction, requiring researchers to perform careful control experiments to verify results.

**258.** (D) Fluorescence Resonance Energy Transfer (FRET)
Fluorescence Resonance Energy Transfer is an advanced optical technique used to monitor protein-protein interactions in living mammalian cells. By labeling two proteins with different fluorophores, researchers can detect when they come into close proximity. If the proteins interact, energy is transferred non-radiatively from the donor to the acceptor fluorophore, providing a dynamic and real-time view of molecular behavior in a natural environment.

**259.** (C) Donor emission spectrum must significantly overlap acceptor excitation.
For efficient FRET to occur between two fluorophores, a critical spectral condition must be met. The emission spectrum of the donor molecule must significantly overlap with the excitation spectrum of the acceptor molecule. This overlap is necessary for the non-radiative transfer of energy to happen. Additionally, the two fluorophores must be positioned very close together, typically within a few nanometers of each other.

**260.** (A) It is typically effective only over distances of 1 to 10 nanometers.
The sensitivity of FRET to distance makes it an excellent "molecular ruler" for studying protein interactions. The effect is typically only efficient over very short distances, generally ranging between 1 and 10 nanometers. This range corresponds perfectly to the dimensions of individual proteins and biological complexes, allowing researchers to determine if two molecules are in direct physical contact within a living cell.

**261.** (D) Phage DNA genotype with displayed surface protein phenotype.
Phage display technology is a powerful tool for screening large libraries of proteins or peptides. Its fundamental principle is the creation of a physical link between the phage's DNA genotype and the displayed surface protein phenotype. By expressing a foreign protein on the surface of the phage particle while keeping the corresponding

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**280.** (B) It eliminates the need for proper experimental controls.
Compensation is a mathematical process in flow cytometry that corrects for the spectral overlap between different fluorophores. It is necessary when using multiple colors simultaneously to ensure accurate data. It is incorrect to claim that compensation eliminates the need for proper experimental controls; in fact, single-stained controls are absolutely essential for accurately calculating the compensation matrix and ensuring the integrity of the results.

**281.** (A) A short, highly structured single-stranded DNA or RNA molecule capable of binding specifically to a target molecule with high affinity
An aptamer is a short, single-stranded nucleic acid molecule, either DNA or RNA, that has been engineered to have a high affinity for a specific target. These molecules fold into complex three-dimensional structures that allow them to bind to a wide range of targets, including proteins, small molecules, and even whole cells, with specificity that rivals that of monoclonal antibodies.

**282.** (A) SELEX (Systematic Evolution of Ligands by Exponential Enrichment)
The isolation of specific aptamers from a vast library of random sequences is achieved through an in vitro selection process called SELEX. This method involves multiple rounds of binding, partitioning, and amplification to enrich for the most effective ligand. By iteratively challenging the library against a target, researchers can identify high-affinity nucleic acid binders for a diverse range of applications in research and diagnostics.

**283.** (A) Aptamers are small, synthetic, and easily modified with tags.
Aptamers offer several advantages over traditional antibodies for use in biosensors. They are synthetic molecules that can be produced entirely in vitro, avoiding the need for animal hosts. Additionally, they are small and can be easily modified with various chemical tags or fluorescent markers. Their high stability and ease of regeneration make them ideal for robust and reproducible diagnostic assays in diverse environments.

**284.** (D) The unbound nucleic acids are washed away, and the bound sequences are eluted.
In a SELEX cycle, the first step is the incubation of the random nucleic acid library with the target molecule. Immediately following this, the mixture is processed to separate the bound sequences from the unbound ones. The unbound nucleic acids are washed away, and the sequences that successfully bound to the target are eluted. These molecules are then amplified to start the next round of selection.

**285.** (A) Contains modified nucleotides to enhance protein binding.
A SOMAmer is a specialized type of aptamer that has been modified to enhance its binding properties. These molecules contain modified nucleotides that introduce protein-like functional groups, such as aromatic rings, into the nucleic acid structure. These modifications lead to a slower dissociation rate (ofF-rate) from the target, resulting in significantly higher binding affinities and improved performance in complex diagnostic applications.

**286.** (A) Regulatory mRNA segment that binds small molecules for control.
A riboswitch is a regulatory segment of a messenger RNA molecule that acts as a natural sensor. It is typically located in the 5' untranslated region and can bind specifically to a small molecule or ligand. This binding induces a conformational change in the mRNA, which then directly controls the gene's expression by influencing processes like transcription termination or translation initiation in cells.

**287.** (D) They suffer from extremely slow reaction times compared to macroscopic assays.
Lab-on-a-chip devices are microfluidic systems that integrate multiple laboratory functions into a single miniature platform. They process tiny fluid volumes and allow for high-throughput analysis. It is incorrect to claim they suffer from slow reaction times. In fact, due to the short diffusion distances and high surface-area-to-volume ratios at the microscale, these devices often achieve much faster reaction times than standard macroscopic assays.

**288.** (A) Polydimethylsiloxane (PDMS)
Polydimethylsiloxane is the most widely used material for fabricating microfluidic devices using soft lithography. It is a transparent, flexible

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**271.** (C) The RNA-Induced Silencing Complex (RISC)
The RNA-Induced Silencing Complex is the functional machinery of the RNAi pathway. It is a multi-protein complex that incorporates the guide strand of an siRNA molecule. Using this sequence as a template, the RISC identifies and binds to complementary target mRNA transcripts. Once bound, the complex's internal endonuclease, Argonaute, cleaves the mRNA, thereby preventing it from being translated into a protein.

**272.** (C) miRNAs are synthetic and never naturally encoded in the host.
siRNAs and miRNAs are both small RNA molecules processed by Dicer that regulate gene expression. siRNAs typically induce direct cleavage, while miRNAs often cause translational repression. It is incorrect to state that miRNAs are synthetic and never naturally encoded. In fact, miRNAs are a fundamental part of the host's natural gene regulation system, encoded within the genome and expressed during development.

**273.** (A) Vector expression allows for stable, long-term gene knockdown.
shRNAs differ from synthetic siRNAs in their delivery and duration of effect. While siRNAs are transiently transfected into cells, shRNAs are typically expressed from a plasmid or viral vector. Once inside the cell, the shRNA is transcribed and then processed by Dicer into functional siRNA. This vector-based approach allows for the stable and long-term knockdown of a target gene in cell lines.

**274.** (B) Targeted delivery of unstable, charged RNA to specific tissues.
The widespread clinical application of RNA interference faces a major challenge in the targeted delivery of the molecules. RNA is highly unstable in the bloodstream and is a large, negatively charged molecule that does not easily cross cell membranes. Successfully delivering functional siRNA to specific tissues without triggering an immune response or ofF-target effects remains the primary hurdle for RNAi therapeutics.

**275.** (A) Approximate cell size or volume
Forward Scatter is a parameter measured in flow cytometry that provides information about the physical characteristics of a cell. As each cell passes through the laser beam, it scatches light at a small angle. The intensity of this scattered light is primarily related to the overall size or volume of the cell, allowing researchers to distinguish between different cell types in a mixture.

**276.** (D) The internal complexity or granularity of the cell
Side Scatter in flow cytometry is measured at a ninety-degree angle to the laser beam. The amount of light scattered at this larger angle is influenced by the internal complexity and granularity of the cell. This includes features like the shape of the nucleus and the presence of cytoplasmic granules, making SSC a valuable tool for identifying different types of white blood cells.

**277.** (A) Isolating live cell populations based on fluorescent parameters.
Fluorescence-Activated Cell Sorting is a specialized type of flow cytometry that allows for the physical isolation of specific cell populations. The system analyzes each cell based on its scatter properties and the intensity of its fluorescent markers. Based on these parameters, the machine can sort individual live cells into different collection tubes, providing a pure population for downstream biological experiments and analysis.

**278.** (D) Propidium Iodide (PI)
Propidium Iodide is the most common intercalating dye used for analyzing the cell cycle by flow cytometry. It binds stoichiometrically to DNA, meaning the amount of dye bound is directly proportional to the DNA content of the cell. Because it cannot cross intact membranes, cells must be fixed or permeabilized before staining, allowing for the precise quantification of DNA throughout the cycle.

**279.** (A) $G_0/G_1$ phase
In a DNA histogram produced by flow cytometry, different peaks represent cells at various stages of the cell cycle. The largest peak, which corresponds to cells with a diploid (2N) DNA content, represents the $G_0$ and $G_1$ phases. These are cells that have not yet started the DNA replication process and therefore have the lowest amount of DNA per cell in the population.

NODIA

**297.** (C) Pacific Biosciences (PacBio) RS/Sequel
Zero-mode waveguides are a key component of Pacific Biosciences (PacBio) sequencing technology. These nanophotonic structures are designed to restrict the observation volume to a tiny space at the bottom of the waveguide. This allows the system to focus on a single DNA polymerase molecule as it incorporates fluorescently labeled nucleotides, enabling the real-time recording of DNA synthesis at the single-molecule level.

**298.** (A) Physical blockage of the pore by nucleobases during translocation.
In nanopore sequencing, the DNA sequence is read by monitoring the ionic current that flows through a protein pore. As a single-stranded DNA molecule translocates through the pore, each individual nucleobase physically blocks the flow of ions in a unique way. These characteristic disruptions in the steady current are recorded and then translated into the DNA sequence using sophisticated algorithms.

**299.** (B) Unwinds dsDNA and controls translocation speed through the pore.
Oxford Nanopore technology utilizes a motor protein to manage the movement of DNA through the pore. The pore itself provides the path for the ionic current, while the motor protein, typically a helicase or polymerase, unwinds the double-stranded DNA and controls the speed at which the single strand translocates. This precise control is essential for the accurate detection of each base.

**300.** (B) Assembly algorithms using long overlaps to average random errors.
Single-molecule long-read sequencing technologies produce very long reads that are valuable for assembly but have higher raw error rates. A critical bioinformatic process for these reads is the use of assembly algorithms that rely on long sequence overlaps. By aligning many overlapping reads, the random errors in the individual sequences can be averaged out, resulting in highly accurate consensus genomic maps.

**********

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reaction
(C) Entropic cost of cellular membrane transport
(D) Enthalpy of protein translation

2015
**59.** Which type of QSAR model explicitly incorporates the three-dimensional spatial orientation and stereochemistry of the molecules?
(A) 1D QSAR
(B) 2D QSAR
(C) 3D QSAR
(D) Topological QSAR

2014
**60.** Select the correct order of sequence database growth relative to computational speed limitations that necessitated heuristic algorithms like FASTA.
(A) Database growth outpaced rigorous dynamic programming speed.
(B) Databases grew linearly while DP scaled exponentially.
(C) Hardware scaled exponentially while databases stayed static.
(D) Heuristic algorithms preceded dynamic programming historically.

2019
**61.** Artificial Neural Networks (ANN) in bioinformatics are structurally modeled to mimic which biological system?
(A) The progressive branching of evolutionary phylogenetic trees
(B) The hierarchical folding patterns of protein domains
(C) The interconnected architecture of biological neurons
(D) The semi-conservative replication of DNA strands

2010
**62.** Which deep learning platform revolutionized structural bioinformatics by predicting protein 3D structures with near-experimental accuracy?
(A) AlphaFold
(B) GeneMark
(C) GROMACS
(D) AutoDock Vina

2008
**63.** Omics data integration in veterinary predictive disease modeling most often combines which distinct data layers?
(A) Topography and climate datasets
(B) Genomics, transcriptomics, and metabolomics
(C) 3D structural coordinates and molecular docking scores
(D) BLAST E-values and FASTA outputs

2015
**64.** Evaluate the following statements regarding the ExPASy Translate tool:
I. It handles both standard and alternative genetic codes.
II. It requires input sequences to be in standard PDB format.
III. It can visually highlight predicted start and stop codons.
IV. It generates structural coordinates of the translated sequence.
Which of the above statement(s) is/are correct?
(A) I and III
(B) II and IV
(C) I, II and III
(D) I, III and IV

2015
**65.** The fundamental concept of the Central Dogma of molecular biology was initially proposed by which scientist?
(A) Rosalind Franklin
(B) James Watson
(C) Francis Crick
(D) Frederick Sanger

2005
**66.** A primary biological database is distinguished by which specific operational policy?
(A) Curators extensively rewrite submission data to ensure uniformity
(B) It archives original experimental data submitted directly by researchers
(C) It exclusively accepts peer-reviewed structural coordinates
(D) It deletes redundant sequence submissions automatically

2017
**67.** The Ensembl Genome Browser is jointly maintained by the European Bioinformatics Institute (EBI) and which other organization?
(A) National Center for Biotechnology Information (NCBI)
(B) Wellcome Trust Sanger Institute
(C) Swiss Institute of Bioinformatics (SIB)
(D) DNA Data Bank of Japan (DDBJ)

NODIA

2012
**68.** Entrez utilizes which specific computational indexing strategy to rapidly retrieve records across its linked databases?

2009

**10.** Match the structural database hierarchies in List I with their correct characteristics in List II.
List I: (Database)
P. SCOP
Q. CATH
R. PDB
S. FSSP
List II: (Characteristic)
1. Purely automated structure comparison using DALI
2. Manual classification based on evolutionary relationships
3. Primary archive of atomic coordinates
4. Semi-automated classification using Class, Architecture, Topology, Homologous superfamily
(A) P-4, Q-2, R-1, S-3
(B) P-2, Q-4, R-3, S-1
(C) P-3, Q-1, R-4, S-2
(D) P-1, Q-3, R-2, S-4

2007

**11.** Which genome browser is characterized by its track-based visual interface and extensive use of BLAT for rapid sequence alignment against reference genomes?
(A) UCSC Genome Browser
(B) NCBI Genome Data Viewer
(C) Ensembl Genome Browser
(D) AnimalQTLdb

2011

**12.** AnimalQTLdb is specifically designed to curate and link genetic markers to which type of data in livestock species?
(A) Three-dimensional protein structures
(B) Post-translational modification sites
(C) Quantitative trait loci and phenotypic traits
(D) Transcription factor binding affinities

2021

**13.** Which boolean query formulation in Entrez will retrieve human p53 records while specifically excluding those related to cancer?
(A) p53 AND human AND cancer
(B) p53 OR human NOT cancer
(C) p53 NOT human OR cancer
(D) p53 AND human NOT cancer

2022

**14.** Which bioinformatics retrieval system was historically developed to link heterogeneous flat-file databases using defined indices, prior to the dominance of Entrez?
(A) DBGET
(B) BioMart
(C) Sequence Retrieval System (SRS)
(D) ExPASy

2017

**15.** The standard FASTA file format requires which specific character to indicate the start of the sequence description line?
(A) ;
(B) >
(C) //
(D) @

2015

**16.** In a GenBank flat file, the end of a complete sequence record is universally denoted by which symbol string?
(A) END
(B)
(C) ||
(D) //

2007

**17.** BioEdit is predominantly utilized by bioinformaticians for which primary application?
(A) Local sequence alignment and manual editing of multiple alignments
(B) Ab initio protein folding prediction
(C) High-throughput genomic read assembly
(D) Automated quantitative structure-activity relationship modeling

2010

**18.** ExPASy Translate tool evaluates how many potential open reading frames for a given double-stranded DNA sequence?
(A) Three
(B) Four
(C) Six
(D) Eight

2013

**19.** The Needleman-Wunsch algorithm employs dynamic programming to achieve which specific type of alignment?
(A) Optimal global alignment
(B) Optimal local alignment
(C) Heuristic multiple alignment
(D) Iterative profile alignment

2009

**20.** Select the correct answer to the following question consisting of Assertion (A) and Reason (R):
**Assertion (A):** The Smith-Waterman algorithm is preferred over Needleman-Wunsch for finding

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2001

**40.** Evaluate the following statements regarding B-cell epitope prediction:

I. Linear epitopes consist of continuous amino acids.

II. Conformational epitopes depend strictly on the primary sequence.

III. Hydrophilicity scales are often used to predict linear B-cell epitopes.

IV. Most natural B-cell epitopes are strictly linear.

Which of the above statement(s) is/are correct?

(A) I and III
(B) I, II and IV
(C) II and III
(D) III and IV

2003

**41.** Reverse vaccinology differs from traditional vaccinology primarily because it initiates the antigen discovery process using:

(A) Live attenuated pathogens cultured in vitro
(B) Whole genomic sequence data analyzed in silico
(C) Purified biochemical extracts from infected host serum
(D) Recombinant plasmids injected into animal models

2016

**42.** Major Histocompatibility Complex (MHC) binding prediction matrices are crucial for identifying which specific immunological target?

(A) B-cell epitopes
(B) T-cell epitopes
(C) Neutralizing antibodies
(D) Macrophage toll-like receptors

2011

**43.** Which machine learning algorithm establishes a hyperplane that maximizes the marginal distance between distinct classification categories?

(A) Artificial Neural Networks
(B) Random Forest
(C) Naive Bayes
(D) Support Vector Machine

2009

**44.** Arrange the sequential steps involved in predicting a protein structure using AlphaFold's deep learning architecture:

I. Multiple sequence alignment generation

II. 3D spatial coordinate generation

III. Feature extraction via transformer neural networks

IV. Input sequence processing

Which of the following is the correct sequence?

(A) IV, I, III, II
(B) IV, III, I, II
(C) I, IV, II, III
(D) I, III, IV, II

NQDIA

2014

**45.** In precision livestock farming, Random Forest algorithms are frequently applied to omics data for which primary purpose?

(A) Discovering de novo protein folds in viral vectors.
(B) Identifying biomarker combinations for trait selection.
(C) Editing targeted genomic loci via CRISPR-Cas9.
(D) Converting raw mass peaks to nucleotide bases.

2003

**46.** The structural architecture of transfer RNA (tRNA) typically folds into which characteristic two-dimensional shape?

(A) Double helix
(B) Cloverleaf
(C) Hairpin loop
(D) Pseudoknot

2017

**47.** Which classification best describes a database that compiles specialized, domain-specific data such as specific metabolic pathways or disease mutations?

(A) Primary database
(B) Generalized sequence archive
(C) Specialized/Boutique database
(D) Composite genomic index

2002

**48.** A bioinformatician downloading complete chromosomal sequences directly from the submitter would access which INSDC database located in Japan?

(A) EMBL
(B) GenBank
(C) DDBJ
(D) TrEMBL

2023

**49.** Match the bioinformatics file formats in List I with their key identifying features in List II.

List I: (File Format)

P. GenBank flat file
Q. EMBL format
R. PDB format
S. FASTA format

calculation → Alignment → Star tree initialization

2006

**30.** Evaluate the following statements regarding Maximum Parsimony:

I. It is a character-based phylogenetic method.
II. It assumes the optimal tree requires the minimum number of evolutionary changes.
III. It is immune to the long-branch attraction artifact.

Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) I only
(D) III only

2009

**31.** Ab initio gene prediction algorithms primarily rely on which genomic features to identify probable coding regions?
(A) Statistical intrinsic signals such as codon bias, splice sites, and start/stop codons
(B) High sequence identity with known cDNA in expressed sequence tag databases
(C) Three-dimensional structural folds of the resultant protein
(D) Synteny conservation across divergent biological species

2024

**32.** Which gene prediction tool is specifically optimized for prokaryotic genomes and relies on inhomogeneous Markov models?
(A) GENSCAN
(B) AUGUSTUS
(C) GeneMark
(D) Fgenesh

2019

**33.** According to Lipinski's Rule of Five, poor oral absorption is likely if the molecular weight of the compound exceeds what specific threshold?
(A) 250 Daltons
(B) 400 Daltons
(C) 500 Daltons
(D) 800 Daltons

2015

**34.** In pharmacokinetics, the acronym ADMET specifies key criteria for drug viability. Which parameter does the 'E' represent?
(A) Efficacy
(B) Elimination
(C) Evaluation
(D) Excretion

2005

**35.** Pharmacophore modeling is typically classified under which Computer-Aided Drug Design (CADD) paradigm?
(A) Ligand-based drug design
(B) Structure-based drug design
(C) De novo structural design
(D) Proteomics-based design

2018

**36.** Which docking approach evaluates multiple conformational states of both the small molecule and the target protein's active site residues?
(A) Rigid body docking
(B) Flexible docking
(C) Blind docking
(D) Pharmacophore docking

2019

**37.** AutoDock utilizes which specific type of search algorithm to explore the conformational space of a ligand during docking?
(A) Dynamic programming
(B) Lamarckian Genetic Algorithm
(C) Maximum Likelihood
(D) Hidden Markov Models

2003

**38.** The primary objective of developing a Quantitative Structure-Activity Relationship (QSAR) model is to:
(A) Correlate compound descriptors with biological activities.
(B) Map 3D coordinate folds of an uncharacterized target protein.
(C) Sequence the exome to locate drug-resistance alleles.
(D) Assemble shotgun genomic reads into contiguous sequences.

2007

**39.** Select the correct answer to the following question consisting of Assertion (A) and Reason (R):

**Assertion (A):** Cross-validation (e.g., leave-one-out) is an essential statistical procedure in 3D QSAR model development.

**Reason (R):** Cross-validation assesses the predictive capability of the model and ensures it is not simply memorizing the training set data.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

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highly conserved domains in largely divergent sequences.

**Reason (R):** Smith-Waterman initializes negative scoring matrix cells to zero, preventing poorly matching regions from degrading the local alignment score.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2011

**21.** Which scoring matrix is computationally derived by extrapolating transition probabilities of amino acid substitutions over evolutionary time?
(A) BLOSUM62
(B) PAM250
(C) Gonnet
(D) JTT

2021

**22.** In affine gap penalty models, why is the gap extension penalty typically set lower than the gap opening penalty?
(A) Standard sequence algorithms cannot process high extension penalties efficiently.
(B) Point mutations are less frequent than structural frameshifts in highly divergent regions.
(C) Biological indels usually occur as single events rather than independent mutations.
(D) Amino acid substitutions require less energy than backbone modifications in peptides.

2002

**23.** An Expect ($E$) value in BLAST indicates the number of distinct alignments expected to occur in a database search by what mechanism?
(A) Directed evolutionary selection
(B) True biological homology
(C) Horizontal gene transfer
(D) Pure random chance

2018

**24.** Which BLAST variant translates a submitted nucleotide query sequence in all six reading frames to search against a protein database?
(A) blastx
(B) blastn
(C) blastp
(D) tblastn

2018

**25.** Which statement represents a disadvantage of the progressive multiple sequence alignment approach used by ClustalW?
(A) It requires excessive computational time for small datasets
(B) Initial alignment errors made at early branching nodes cannot be corrected later
(C) It relies entirely on structural coordinates rather than sequence
(D) It is incapable of processing more than three sequences simultaneously

2016

**26.** T-Coffee improves upon standard progressive alignment by incorporating which specific mathematical framework?
(A) Hidden Markov Models
(B) A library of pairwise global and local alignments
(C) Position-Specific Scoring Matrices
(D) Artificial Neural Networks

2007

**27.** Homologous genes separated by a speciation event that typically retain the same biological function are defined as:
(A) Orthologs
(B) Paralogs
(C) Xenologs
(D) Analogs

NODIA

2005

**28.** In molecular phylogeny, what structural feature structurally distinguishes a cladogram from a phylogram?
(A) A cladogram lacks a root node entirely
(B) Cladograms can only be constructed using character-based methods
(C) Branch lengths in a cladogram do not represent evolutionary time or amount of character change
(D) A cladogram requires sequence alignments containing exclusively DNA

2010

**29.** Select the correct sequence of computational steps involved in constructing a phylogenetic tree using the Neighbor-Joining method.
(A) Alignment → Distance matrix calculation → Star tree initialization → Node consolidation
(B) Distance matrix calculation → Alignment → Node consolidation → Star tree initialization
(C) Star tree initialization → Alignment → Node consolidation → Distance matrix calculation
(D) Node consolidation → Distance matrix

List II: (Feature)
1. Two-letter line type codes (e.g., ID, SQ)
2. ATOM and HETATM coordinate records
3. FEATURES table and ORIGIN block
4. Single line starting with ">" followed by raw sequence

(A) P-3, Q-1, R-2, S-4
(B) P-1, Q-3, R-4, S-2
(C) P-4, Q-2, R-1, S-3
(D) P-2, Q-4, R-3, S-1

2011

**50.** Evaluate the following statements on sequence alignment scoring:
I. PAM matrices are based on observed mutations in closely related proteins.
II. BLOSUM matrices are based on highly conserved blocks in diverse alignments.
III. PAM250 is suitable for comparing highly divergent sequences.
IV. BLOSUM62 is the default matrix for blastp.
V. A high PAM number correlates to a low BLOSUM number.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, II, III, IV and V
(C) II, III and V
(D) I, III, IV and V

2025

**51.** The primary advantage of heuristic database search algorithms like BLAST over dynamic programming is:
(A) Substantially decreased computational time
(B) Guaranteed optimal alignment accuracy
(C) Elimination of the need for substitution matrices
(D) Ability to perfectly align structural domains

2009

**52.** Which BLAST program translates a nucleotide database in all six reading frames and compares it against a translated nucleotide query?
(A) tblastn
(B) tblastx
(C) blastx
(D) blastn

2021

**53.** Progressive multiple sequence alignment tools build the final alignment guided by which preliminary computational structure?
(A) Hidden Markov Model
(B) Guide tree
(C) Artificial neural network
(D) Position-specific scoring matrix

2012

**54.** Evaluate the following phylogenetic statements:
I. UPGMA assumes a constant rate of molecular evolution (molecular clock).
II. Neighbor-Joining does not assume a constant rate of evolution.
III. Maximum Parsimony is computationally faster than UPGMA for large datasets.
Which of the above statement(s) is/are INCORRECT?
(A) I and II
(B) II and III
(C) III only
(D) I only

2003

**55.** Homology-based gene prediction relies critically on which underlying biological assumption?
(A) Genes from related organisms share statistically identical GC content
(B) Conserved protein sequences imply conserved genomic coding regions
(C) Splice sites are absolutely identical across all eukaryotic domains
(D) Pseudogenes retain identical promoter sequences to active genes

NODIA

2012

**56.** Which of the following is NOT a parameter evaluated in Lipinski's Rule of Five?
(A) Number of hydrogen bond donors
(B) Number of hydrogen bond acceptors
(C) Calculated $\log P$ (lipophilicity)
(D) Number of rotatable bonds

2015

**57.** Structure-based drug design requires which prerequisite biological data to initiate the modeling process?
(A) A library of known active small-molecule ligands
(B) The 3D coordinate structure of the target macromolecule
(C) High-throughput genomic sequences of the pathogen
(D) Phenotypic microarray expression data

2007

**58.** In molecular docking, the scoring function is fundamentally designed to estimate which thermodynamic property?
(A) Binding free energy between the ligand and the receptor
(B) Activation energy of the target's enzymatic

(B) Support Vector Machines
(C) Inhomogeneous Markov chain models
(D) Pearson correlation coefficients

2018
**77.** Which molecular descriptor refers to the predicted partition coefficient of a compound between octanol and water?
(A) $\log P$
(B) Polar surface area
(C) Molar refractivity
(D) pKa

2012
**78.** In ligand-based drug design, the term 'pharmacophore' is best defined as:
(A) A spatial arrangement of structural features essential for a molecule's biological activity
(B) The geometric arrangement of atoms in a receptor binding site
(C) The minimum sequence of amino acids required for drug binding
(D) The specific metabolic pathway responsible for drug degradation

2007
**79.** Match the molecular docking algorithms in List I with their brief descriptions in List II.
List I: (Algorithm)
P. Monte Carlo
Q. Genetic Algorithm
R. Simulated Annealing
S. Incremental Construction
List II: (Description)
1. Recombines spatial features of successful ligand poses to generate new poses
2. Randomly alters ligand conformation and accepts it based on a probability threshold
3. Docks small fragments of a ligand sequentially into the active site
4. Uses a gradual 'cooling' parameter to escape local energy minima
(A) P-2, Q-1, R-4, S-3
(B) P-1, Q-2, R-3, S-4
(C) P-4, Q-3, R-1, S-2
(D) P-3, Q-4, R-2, S-1

2019
**80.** Select the correct answer to the following question consisting of Assertion (A) and Reason (R):
**Assertion (A):** 3D-QSAR requires structural alignment (superposition) of the training set molecules before calculating interaction fields.
**Reason (R):** Descriptors in 3D-QSAR are spatially dependent and are evaluated on a 3D grid surrounding the aligned molecules.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2005
**81.** In reverse vaccinology, which in silico screening criterion is critical for avoiding autoimmune responses?
(A) Ensuring the antigen shares high homology with host proteins
(B) Excluding antigens that show homology to host proteins
(C) Selecting exclusively non-structural viral proteins
(D) Targeting deeply buried hydrophobic protein cores

2001
**82.** Which immunoinformatics tool is widely used to predict the binding affinity of peptide sequences to human leukocyte angle (HLA) molecules?
(A) NetMHC
(B) ClustalW
(C) AutoDock
(D) Glimmer

2021
**83.** The Random Forest machine learning algorithm classifies data by constructing an ensemble of which structures?
(A) Artificial neural networks
(B) Hidden Markov models
(C) Decision trees
(D) Support vector hyperplanes

2010
**84.** In veterinary bioinformatics, predictive disease modeling often incorporates SNP chip data. What does SNP stand for?
(A) Single Nucleotide Polymorphism
(B) Structural Node Protein
(C) Sequence Number Parameter
(D) Synteny Null Point

2002
**85.** During post-transcriptional modification in eukaryotes, which structure is added to the 3-prime end of mRNA?
(A) 7-methylguanosine cap
(B) Poly-A tail
(C) Spliceosome complex

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(A) Hash table dynamic programming
(B) Boolean logic applied to pre-computed inverted indices
(C) Real-time Smith-Waterman alignment parsing
(D) Hidden Markov Model profile scanning

2007

**69.** Select the correct answer to the following question consisting of Assertion (A) and Reason (R):
**Assertion (A):** The EMBL flat file format utilizes two-letter line codes to denote specific data fields.
**Reason (R):** This standardized line coding enables parsers to rapidly extract specific metadata like accession numbers and species origin without reading the entire file.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2008

**70.** Match the sequence alignment software in List I with its defining methodology in List II.
List I: (Software)
P. Clustal Omega
Q. MUSCLE
R. BLAST
S. HMMER
List II: (Methodology)
1. Heuristic pairwise alignment based on k-mer seeding
2. Profile hidden Markov models for sequence searches
3. Iterative multiple sequence alignment using log-expectation scores
4. Scalable progressive multiple sequence alignment using guide trees
(A) P-4, Q-3, R-1, S-2
(B) P-3, Q-4, R-2, S-1
(C) P-1, Q-2, R-3, S-4
(D) P-2, Q-1, R-4, S-3

2025

**71.** In pairwise sequence alignment, an affine gap penalty applies a higher penalty to which specific event?
(A) Opening a new gap
(B) Extending an existing gap
(C) Mismatching two polar amino acids
(D) Matching identical nucleotides

2014

**72.** Which FASTA program is designed to search a translated DNA sequence database against a protein query?
(A) fasta
(B) fastx
(C) tfasta
(D) tfasty

2024

**73.** Which consequence most accurately describes the 'once a gap, always a gap' rule in standard progressive multiple sequence alignment?
(A) Early gap insertion errors are not removed in later stages.
(B) Terminal gaps are ignored in sequence scoring calculations.
(C) Affine gap penalties are disabled for divergent sequences.
(D) Iterative refinement algorithms cannot be applied here.

NODIA

2014

**74.** Evaluate these statements about evolutionary tree roots:
I. An unrooted tree indicates the evolutionary relationships among taxa without specifying a common ancestor.
II. A rooted tree provides directionality to evolutionary time.
III. Outgroup selection is a common method to root an unrooted tree.
IV. Unrooted trees contain more nodes than their rooted counterparts for the same taxa.
V. UPGMA intrinsically produces a rooted tree.
Which of the above statement(s) is/are correct?
(A) I, II, III and V
(B) I, II, III and IV
(C) II, III, IV and V
(D) I, III, IV and V

2017

**75.** Maximum Likelihood phylogenetic construction primarily differs from Maximum Parsimony by utilizing:
(A) Distance matrices to construct star trees
(B) An explicit probabilistic model of sequence evolution
(C) Purely phenotypic character states
(D) Heuristic blastn alignments

2001

**76.** The GeneMark algorithm predicts coding regions by utilizing which specific mathematical model?
(A) Affine gap penalties

# CHAPTER 08
# Bioinformatics in Biotechnology

2002

**1.** Which molecular process describes the direct flow of genetic information from RNA to DNA in specific viral life cycles?
(A) Reverse transcription
(B) Splicing
(C) Translation
(D) Replication

2022

**2.** The structural distinction between RNA and DNA primarily relies on the presence of which chemical group at the 2-prime position of the pentose sugar?
(A) Methyl group
(B) Phosphate group
(C) Hydroxyl group
(D) Amino group

2023

**3.** Which of the following represents a composite biological database that integrates data from multiple primary sources without storing original submissions?
(A) OWL
(B) GenBank
(C) Protein Data Bank
(D) Sequence Retrieval System

2016

**4.** In the classification of biological databases, which statement accurately reflects the function of a secondary database?
(A) It archives original experimental sequence data directly from researchers
(B) It provides unannotated raw genomic reads from sequencing platforms
(C) It exclusively houses three-dimensional coordinate files of nucleic acids
(D) It stores computationally derived consensus patterns, motifs, and profiles

2010

**5.** The International Nucleotide Sequence Database Collaboration (INSDC) ensures daily data synchronization among which three primary databases?
(A) GenBank, UniProt, and PDB
(B) EMBL, DDBJ, and GenBank
(C) RefSeq, Ensembl, and UCSC
(D) Swiss-Prot, TrEMBL, and PIR

2011

**6.** A researcher attempting to submit a newly sequenced annotated gene to EMBL should utilize which specific submission protocol or tool?
(A) BankIt
(B) Sequin
(C) Webin
(D) Sakura

2001

**7.** Which section of UniProtKB contains high-quality, manually annotated, and non-redundant protein sequence records?
(A) TrEMBL
(B) PIR-PSD
(C) UniParc
(D) Swiss-Prot

2022

**8.** The Protein Information Resource (PIR) was originally developed to support which specific bioinformatics task?
(A) Identifying homologous proteins based on evolutionary superfamilies
(B) Archiving 3D structural coordinates of crystallized proteins
(C) Storing raw mass spectrometry peptide output
(D) Predicting ab initio gene locations in eukaryotic genomes

2011

**9.** Identify the INCORRECT statement regarding the Protein Data Bank (PDB).
(A) It is the primary repository for experimentally determined 3D structures
(B) It accepts structural data generated exclusively by X-ray crystallography
(C) PDB files contain atomic coordinates and experimental metadata
(D) Each entry is assigned a unique four-character alphanumeric identifier

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(D) Ribosomal binding site

2008

**86.** Which primary sequence database exclusively curates sequence data submitted directly by Japanese researchers but shares it globally?
(A) EMBL
(B) DDBJ
(C) PDB
(D) GenBank

2009

**87.** The Swiss-Prot database differentiates itself from TrEMBL by emphasizing which specific feature?
(A) Automated translation of structural RNAs
(B) Manual literature-based curation and biological annotation
(C) High-throughput genomic read archiving
(D) De novo protein structure prediction

2017

**88.** CATH classification organizes protein structures based on which four hierarchical levels?
(A) Core, Alpha-helix, Turn, Homology
(B) Conformation, Alignment, Threading, Homology
(C) Class, Architecture, Topology, Homologous superfamily
(D) Class, Alignment, Topology, Helix

2014

**89.** Select the sequence of databases ordered correctly from primary repository to secondary motif database to structural database.
(A) GenBank → PROSITE → PDB
(B) PROSITE → PDB → GenBank
(C) PDB → GenBank → PROSITE
(D) GenBank → PDB → PROSITE

2021

**90.** Arrange the workflow for submitting a novel sequence to GenBank via BankIt:
I. Upload sequence data in FASTA format
II. Provide author and publication metadata
III. Receive GenBank accession number
IV. Add biological annotations and feature keys
Which of the following is the correct sequence?
(A) II, I, IV, III
(B) I, II, III, IV
(C) II, IV, I, III
(D) IV, I, II, III

2003

**91.** In a standard PDB coordinate file, which record type specifically lists the atomic coordinates for standard amino acid residues?
(A) ATOM
(B) HETATM
(C) SEQRES
(D) HEADER

2022

**92.** The Sequence Retrieval System (SRS) utilizes which data structure to facilitate rapid querying across multiple flat-file databases?
(A) Distance matrices
(B) Affine gap models
(C) Inverted indexes
(D) Convolutional neural networks

2024

**93.** Open Reading Frame (ORF) Finder tools scan DNA sequences for ORFs bounded by which specific functional codons?
(A) Start codons and Stop codons
(B) Start codons and Splice sites
(C) Promoter sequences and Poly-A signals
(D) Shine-Dalgarno sequences and TATA boxes

2015

**94.** The Smith-Waterman algorithm guarantees mathematically optimal results for which type of alignment?
(A) Local sequence alignment
(B) Heuristic global alignment
(C) Multiple sequence alignment
(D) Profile-profile alignment

2013

**95.** The BLOSUM62 matrix was derived from sequence blocks sharing what specific threshold of amino acid identity?
(A) Less than or equal to 62% identity
(B) Greater than or equal to 62% identity
(C) Exactly 62% identity
(D) Precisely 62 distinct residue matches

NODIA

2019

**96.** If a BLAST search returns an $E$-value of 0.0, what does this indicate about the alignment?
(A) The alignment is entirely random
(B) The match is statistically highly significant and extremely unlikely to be due to chance
(C) The database contains zero homologous sequences
(D) The alignment score was zero

2019

**97.** Which multiple sequence alignment program utilizes an iterative approach specifically optimizing a log-expectation objective function?
(A) MUSCLE

2014

**136.** The Central Dogma emphasizes that information cannot flow back from which macromolecule to nucleic acids?
(A) DNA
(B) mRNA
(C) Protein
(D) tRNA

2025

**137.** Which database represents a specialized resource for protein sequences derived exclusively from the translation of all coding sequences in GenBank, EMBL, and DDBJ?
(A) PDB
(B) CATH
(C) RefSeq
(D) GenPept

2023

**138.** The submission of raw, unaligned next-generation sequencing reads is typically directed to which specific NCBI archive?
(A) Gene Expression Omnibus (GEO)
(B) Protein Data Bank (PDB)
(C) dbSNP
(D) Sequence Read Archive (SRA)

2022

**139.** Match the bioinformatics tools/databases in List I with their primary domain in List II.
List I: (Tool/Database)
P. Swiss-Prot
Q. AlphaFold
R. Clustal Omega
S. GeneMark
List II: (Domain)
1. Protein structure prediction
2. Curated protein sequences
3. Ab initio gene prediction
4. Multiple sequence alignment
(A) P-2, Q-1, R-4, S-3
(B) P-1, Q-2, R-3, S-4
(C) P-3, Q-4, R-1, S-2
(D) P-4, Q-3, R-2, S-1

2009

**140.** Evaluate the following statements about CATH:
I. 'C' stands for Class, determined by secondary structure content.
II. 'A' stands for Architecture, describing the macroscopic arrangement of secondary structures.
III. 'T' stands for Topology, which considers sequence connectivity.
IV. It relies exclusively on manual curation without automated algorithms.
Which of the above statement(s) is/are correct?
(A) I, II and III
(B) I, II and IV
(C) II, III and IV
(D) I and III

2001

**141.** The AnimalQTLdb is an invaluable resource for veterinary bioinformaticians primarily aiming to map which type of traits?
(A) Monogenic lethal mutations in rare pedigree lines.
(B) Polygenic traits like milk yield and disease resistance.
(C) Viral integrase binding sites in host chromosomes.
(D) Prion protein folding pathways in animal models.

2018

**142.** Entrez allows researchers to utilize 'Limits' or 'Filters'. What is the primary computational benefit of this feature?
(A) It deletes unwanted data from the global repository.
(B) It translates queries into Japanese for DDBJ servers.
(C) It narrows search space to improve result relevance.
(D) It builds a phylogenetic tree from search results.

2025

**143.** Which standardized file format strictly represents three-dimensional atomic coordinates and is heavily utilized in molecular docking?
(A) FASTA
(B) PDB
(C) GenBank flat file
(D) FASTQ

2016

**144.** Arrange the correct procedural sequence for performing an effective homology-based BLAST search:
I. Select appropriate BLAST variant (e.g., blastp)
II. Interpret E-values and bit scores
III. Input query sequence in FASTA format
IV. Choose target database (e.g., nr)
Which of the following is the correct sequence?
(A) III, I, IV, II
(B) I, III, IV, II
(C) III, IV, I, II

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(B) Deep Learning
(C) K-Nearest Neighbors
(D) Principal Component Analysis

2014

**108.** The integration of genomic data into animal breeding programs to predict the breeding value of an animal without relying solely on phenotype is termed:
(A) Genomic selection
(B) Epigenetic tracing
(C) Clonal propagation
(D) Proteomic pedigree

2018

**109.** Match the bioinformatics databases in List I with their specific data focus in List II.
List I: (Database)
P. NCBI Genome
Q. SCOP
R. PIR
S. TrEMBL
List II: (Focus)
1. Unreviewed, computationally translated protein sequences
2. Highly curated protein superfamily classifications
3. Structural classifications based purely on evolutionary relationships
4. Complete chromosome and assembly metadata
(A) P-4, Q-3, R-2, S-1
(B) P-3, Q-4, R-1, S-2
(C) P-1, Q-2, R-4, S-3
(D) P-2, Q-1, R-3, S-4

2021

**110.** Which molecular process accurately defines the synthesis of RNA from a DNA template?
(A) Replication
(B) Translation
(C) Reverse transcription
(D) Transcription

2013

**111.** Primary sequence databases rely on international collaboration. The INSDC comprises GenBank, EMBL, and DDBJ. Which statement correctly identifies their respective host regions?
(A) USA, Europe, Japan
(B) USA, UK, China
(C) Canada, Europe, Japan
(D) Europe, Australia, Japan

2006

**112.** The TrEMBL database was explicitly created to overcome which bottleneck in bioinformatics?
(A) The unable to store massive 3D coordinate files
(B) The time lag caused by the slow, manual curation process required for Swiss-Prot
(C) The lack of algorithms for multiple sequence alignment
(D) The limitation of standard FASTA formatting

2001

**113.** Which structural database manually organizes proteins into Families, Superfamilies, and Folds based on detailed visual inspection of evolutionary relationships?
(A) CATH
(B) PDB
(C) SCOP
(D) FSSP

NODIA

2002

**114.** Evaluate the following statements regarding genome browsers:
I. UCSC Genome Browser uses a track-based interface.
II. Ensembl provides extensive annotation for vertebrate genomes.
III. AnimalQTLdb contains structural coordinates of animal viruses.
IV. NCBI Genome Data Viewer allows visualization of sequence alignments.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, II and III
(C) II, III and IV
(D) I, III and IV

2015

**115.** A researcher utilizing the Entrez database interface is fundamentally executing a search across:
(A) A federated network of distinctly maintained life science databases
(B) A single, monolithic SQL database of nucleotide sequences
(C) Only peer-reviewed publications within PubMed
(D) Exclusively three-dimensional protein structures

2004

**116.** In the FASTA format, a sequence line containing the character "N" indicates:
(A) A structural gap in the alignment

(B) ClustalW
(C) T-Coffee
(D) MAFFT

2002

**98.** In molecular phylogeny, a grouping that includes a single common ancestor and all of its descendants is strictly termed:
(A) Monophyletic
(B) Paraphyletic
(C) Polyphyletic
(D) Homoplastic

2006

**99.** Select the correct answer to the following question consisting of Assertion (A) and Reason (R):
**Assertion (A):** Distance-based phylogenetic methods compress sequence data into a single numerical distance value between taxa.
**Reason (R):** This compression prevents distance methods from tracking the evolutionary history of specific, individual nucleotide changes.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2024

**100.** Glimmer is widely used for predicting genes primarily in which type of organisms?
(A) Mammals
(B) Plants
(C) Fungi
(D) Bacteria and Archaea

2021

**101.** The ADMET property 'T' represents which pharmacological concept essential for drug viability?
(A) Toxicity
(B) Transport
(C) Translation
(D) Thermodynamics

2012

**102.** Structure-based drug design typically assumes which physical relationship between a drug and its target?
(A) The drug exerts its effect by structurally binding to a specific pocket on the target
(B) The drug chemically digests the target protein
(C) The drug alters the target's gene expression at the DNA level
(D) The drug acts as a non-specific chaotropic agent

2012

**103.** In molecular docking, a 'rigid body' approach implies that:
(A) Only the receptor side chains are allowed to move
(B) The ligand relies on covalent bonds to secure the pose
(C) Neither the ligand nor the receptor can change conformation during the docking simulation
(D) Water molecules are tightly bound in the active site

2009

**104.** Evaluate the following statements regarding QSAR validation:
I. R-squared ($R^2$) measures the goodness of fit of the training data.
II. Q-squared ($Q^2$) measures the predictive capability using cross-validation.
III. A high $R^2$ guarantees a high $Q^2$.
Which of the above statement(s) is/are correct?
(A) I and II
(B) I and III
(C) II and III
(D) I, II and III

2021

**105.** B-cell epitopes that rely on the folded, tertiary structure of a protein to bring distant amino acids into spatial proximity are known as:
(A) Conformational epitopes
(B) Linear epitopes
(C) Cryptic epitopes
(D) T-cell dependent epitopes

2023

**106.** The foundational concept of predicting immunogenic T-cell epitopes relies heavily on modeling the peptide's affinity to which host cell structure?
(A) Toll-like receptors
(B) CD4 coreceptors
(C) MHC / HLA complexes
(D) T-cell receptor hypervariable regions

2014

**107.** Which machine learning approach processes data in multiple layers of abstractions, mimicking deep neural architectures, and is extensively used in AlphaFold?
(A) Support Vector Machines

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2005

**127.** According to Lipinski's Rule of Five, a drug candidate should ideally have no more than how many hydrogen bond donors?
(A) 5
(B) 10
(C) 15
(D) 20

2001

**128.** In Computer-Aided Drug Design (CADD), fragment-based drug design typically starts by identifying compounds with which relative characteristic?
(A) Low molecular weight and low binding affinity
(B) High molecular weight and low binding affinity
(C) High molecular weight and high binding affinity
(D) Low molecular weight and high binding affinity

2010

**129.** Select the correct answer to the following question consisting of Assertion (A) and Reason (R):
**Assertion (A):** Flexible ligand docking is computationally more intensive than rigid body docking.
**Reason (R):** Flexible docking must evaluate multiple torsional rotations and conformational states of the ligand within the active site.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2008

**130.** Evaluate the following regarding scoring functions in molecular docking:
I. Empirical scoring functions use terms based on binding energy components like hydrogen bonds and hydrophobics.
II. Knowledge-based scoring functions derive potentials from statistical analysis of crystal structures.
III. Force-field methods ignore electrostatic interactions entirely.
Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I only

2012

**131.** The primary purpose of calculating molecular descriptors in QSAR is to:
(A) Convert chemical structures into mathematical variables suitable for statistical modeling
(B) Animate the molecular dynamics of a protein-ligand complex
(C) Predict the species origin of a small molecule
(D) Align distinct compound sequences using dynamic programming

2001

**132.** In silico T-cell epitope prediction frequently relies on position-specific scoring matrices (PSSMs) derived from:
(A) Known viral capsids
(B) Experimentally validated MHC-binding peptides
(C) Whole bacterial genomes
(D) Antibody hypervariable regions

2009

**133.** Which algorithm represents an ensemble machine learning method that builds multiple decision trees during training and outputs the mode of the classes?
(A) Artificial neural networks
(B) Hidden Markov models
(C) Random Forest
(D) Support vector hyperplanes

2006

**134.** Which application represents a direct use of Artificial Neural Networks (ANN) in transcriptomics?
(A) Classifying disease states based on complex gene expression microarray patterns
(B) Assembling short genomic reads into contigs
(C) Formatting data files into standard FASTA
(D) Calculating exact Needleman-Wunsch gap extensions

2003

**135.** Precision livestock farming utilizes bioinformatics and sensor data to optimize which of the following?
(A) In vitro fertilization rates in laboratory mice
(B) De novo design of synthetic veterinary antibiotics
(C) Real-time monitoring and management of animal health, welfare, and production
(D) Sequencing of extinct livestock ancestors

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(A) BioEdit
(B) ExPASy Proteomics Server
(C) AutoDock
(D) GeneMark

2024
**176.** Which dynamic programming algorithm is utilized to find the optimal global alignment across the entire lengths of two sequences?
(A) Smith-Waterman
(B) BLAST
(C) Needleman-Wunsch
(D) FASTA

2021
**177.** A PAM matrix is derived by analyzing sequences that share at least what percentage of global identity?
(A) 15%
(B) 40%
(C) 62%
(D) 85%

2018
**178.** $E$-values in BLAST are directly proportional to which specific search parameter?
(A) The number of mismatches allowed
(B) The physical size of the search database
(C) The percentage of GC content in the query
(D) The substitution matrix used

2021
**179.** A researcher wants to compare a newly sequenced novel protein against an unannotated whole-genome sequence. Which BLAST program is most appropriate?
(A) blastp
(B) tblastn
(C) blastx
(D) blastn

2006
**180.** Select the correct order of operational steps executed by the ClustalW progressive alignment algorithm:
(A) Compute pairwise distance matrix → Construct guide tree → Perform progressive multiple alignment
(B) Construct guide tree → Compute pairwise distance matrix → Perform progressive multiple alignment
(C) Perform progressive multiple alignment → Construct guide tree → Compute pairwise distance matrix
(D) Compute pairwise distance matrix → Perform progressive multiple alignment → Construct guide tree

2012
**181.** In molecular phylogeny, how is an outgroup defined?
(A) A taxon unrelated to the ingroup and randomly chosen.
(B) A taxon diverged prior to the ingroup's common ancestor.
(C) The most recently evolved species in a specific clade.
(D) A hybrid species connecting two distinct lineages.

2010
**182.** Which phylogenetic construction methodology evaluates distinct nucleotide positions individually rather than relying on an aggregated distance metric?
(A) Maximum Parsimony
(B) UPGMA
(C) Neighbor-Joining
(D) Jukes-Cantor modeling

2008
**183.** Identify the principal limitation of the UPGMA phylogenetic tree construction method.
(A) It requires protein coordinates
(B) It explicitly assumes a strict molecular clock across all lineages
(C) It cannot process unaligned sequences
(D) It inherently produces unrooted trees

2025
**184.** Homology-based gene prediction tools frequently utilize spliced alignments. Which software is highly recognized for aligning cDNA transcripts to genomic DNA to identify exon structures?
(A) BLASTP
(B) AutoDock Vina
(C) Spidey
(D) NetMHC

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2007
**185.** According to Lipinski's Rule of Five, an ideal oral drug candidate should possess a calculated $\log P$ (clogP) value not exceeding:
(A) 2
(B) 5
(C) 10
(D) 500

2010
**186.** Which methodology attempts to construct a novel drug molecule explicitly from scratch within the constraints of the target active site?

(D) I, IV, III, II

2025

**145.** Select the correct answer to the following question consisting of Assertion (A) and Reason (R):

**Assertion (A):** The ExPASy Translate tool often yields six distinct translation frames for a given DNA sequence.

**Reason (R):** Double-stranded DNA possesses three forward reading frames on the sense strand and three reverse reading frames on the antisense strand.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2022

**146.** In sequence alignment, the bit score is fundamentally distinguished from the raw score because it is:

(A) Dependent on the size of the database
(B) Normalized to be independent of the specific scoring matrix used
(C) Calculated exclusively for nucleotide sequences
(D) A direct measure of evolutionary time

2004

**147.** A BLOSUM45 matrix is mathematically tailored to align sequences that are:

(A) Highly identical (recent evolutionary divergence)
(B) Moderately identical
(C) Highly divergent (distant evolutionary relationships)
(D) From the exact same species

2025

**148.** In the context of sequence alignments, which definition accurately describes a "gap"?

(A) A mismatch between two identical purines
(B) An introduced space representing an evolutionary insertion or deletion event
(C) An unsequenced region of a chromosome
(D) The termination of a translation event

2002

**149.** The FASTA algorithm uniquely identifies regions of absolute identity between two sequences before performing dynamic programming. What are these exact matches called?

(A) Contigs
(B) $K$-tuples or words
(C) Pharmacophores
(D) Hidden states

2005

**150.** Evaluate the following statements on Multiple Sequence Alignment (MSA):

I. Iterative methods refine the initial alignment by repeatedly realigning subgroups.
II. MUSCLE utilizes $k$-mer counting for rapid distance estimation.
III. Progressive alignments are mathematically guaranteed to find the absolute optimal MSA.
IV. Clustal Omega can handle alignments of hundreds of thousands of sequences using Hidden Markov Models.
V. MSA is a prerequisite for reliable phylogenetic tree construction.

Which of the above statement(s) is/are correct?

(A) I, II, IV and V
(B) I, II, III and V
(C) II, III, IV and V
(D) I, III, IV and V

2017

**151.** Homologous genes acquired by a genome through horizontal gene transfer between different species are defined as:

(A) Orthologs
(B) Paralogs
(C) Xenologs
(D) Analogs

2007

**152.** Distance-based phylogenetic construction algorithms include which two widely utilized methods?

(A) Maximum Parsimony and Maximum Likelihood
(B) UPGMA and Neighbor-Joining
(C) Needleman-Wunsch and Smith-Waterman
(D) Hidden Markov Models and Neural Networks

2023

**153.** A phylogenetic tree algorithm infers a tree that maximizes the probability of observing the given sequence alignment under a specific model of sequence evolution. This describes:

(A) Neighbor-Joining
(B) Maximum Likelihood
(C) Maximum Parsimony
(D) Bootstrapping

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(B) The end of the sequence record
(C) An ambiguous or unknown nucleotide
(D) A stop codon

2014
**117.** Sequin was a widely used standalone software tool designed primarily for:
(A) Preparing complex sequence submissions for GenBank
(B) Aligning highly divergent protein sequences
(C) Predicting 3D structures from primary sequences
(D) Performing genome-wide association studies

2009
**118.** The dynamic programming matrix for a global sequence alignment initializes the first row and column by:
(A) Accumulating gap penalties linearly
(B) Setting all values to zero
(C) Assigning random positive integers
(D) Copying the sequence weights directly

2021
**119.** A PAM1 substitution matrix models evolutionary divergence corresponding to what specific metric?
(A) 1 million years of evolutionary time
(B) 1 accepted point mutation per 100 amino acids
(C) 1 percent similarity between sequence blocks
(D) 1 base pair insertion per gene

2010
**120.** Which feature mathematically defines an affine gap penalty function, where $G$ is the total penalty, o is the gap open penalty, $e$ is the extension penalty, and $L$ is gap length?
$G = o + e \times L$
(A) $G = o + e \times L$
(B) $G = o \times e \times L$
(C) $G = e + o \times L$
(D) $G = o \times L$

2023
**121.** The BLAST heuristic algorithm begins its search by performing which computational step?
(A) Constructing a full Needleman-Wunsch matrix
(B) Identifying short, exact word matches (seeds) between query and database
(C) Aligning the entire sequence using a guide tree
(D) Calculating the GC content of the database

2009
**122.** T-Coffee builds an initial library of pairwise alignments using both global and local alignment data. What is the primary advantage of this approach?
(A) It improves accuracy for globally divergent sequences.
(B) It reduces computational time compared to ClustalW.
(C) It eliminates the need for scoring matrices.
(D) It constructs a Maximum Likelihood tree.

2007
**123.** Genes resulting from a gene duplication event within a single lineage that may evolve new functions are specifically designated as:
(A) Orthologs
(B) Homoplasies
(C) Paralogs
(D) Synapomorphies

2001
**124.** Evaluate the following statements regarding the Neighbor-Joining (NJ) method:
I. It is a character-based method.
II. It constructs unrooted trees.
III. It attempts to minimize the total sum of branch lengths at each stage.
IV. It is statistically robust for large datasets.
V. It assumes a strict molecular clock.
Which of the above statement(s) is/are correct?
(A) II, III and IV
(B) I, II and III
(C) II, IV and V
(D) III, IV and V

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2013
**125.** Which phenomenon frequently causes Maximum Parsimony to infer incorrect phylogenetic trees when certain branches have evolved exceptionally rapidly?
(A) Transition/transversion bias
(B) Long-branch attraction
(C) Codon usage bias
(D) Incomplete lineage sorting

2017
**126.** In eukaryotic gene prediction, Ab initio algorithms must accurately model which unique structural feature absent in prokaryotes?
(A) Exon-intron boundaries (splice sites)
(B) Promoter regions
(C) Stop codons
(D) Ribosome binding sites

(A) Fourier transforms
(B) Kernel trick
(C) Affine gap functions
(D) Log-odds ratios

2021

**165.** The AlphaFold architecture drastically improves 3D structure prediction by utilizing which specific type of deep learning module?
(A) Evoformer (Transformer-based architecture)
(B) Simple Multilayer Perceptron
(C) K-Means clustering algorithm
(D) Decision Tree classifier

2017

**166.** In precision livestock farming, how does the integration of genomic selection models impact generation intervals?
(A) It significantly decreases the generation interval
(B) It significantly decreases the generation interval
(C) It has no effect on the generation interval
(D) It replaces sexual reproduction entirely

2005

**167.** Which molecular process represents an exception to the classical Central Dogma by demonstrating RNA selF-replication without a DNA intermediate?
(A) RNA virus replication via RNA-dependent RNA polymerase
(B) Retroviral integration
(C) Spliceosome editing
(D) Ribosomal translation

2007

**168.** Which biological database structure natively supports rapid complex queries and minimizes data redundancy through relational tables?
(A) Flat file databases
(B) Relational databases
(C) Hierarchical archives
(D) Object-oriented text files

2017

**169.** What is the defining characteristic of a composite biological database?
(A) It aggregate and cross-references data from multiple distinct primary and secondary databases
(B) It generates purely synthetic nucleotide sequences
(C) It hosts physical biological tissue samples
(D) It archives raw electron microscopy images

2004

**170.** Which specific feature identifies a TrEMBL record compared to a Swiss-Prot record within UniProt?
(A) TrEMBL records lack sequence data and store interactions.
(B) TrEMBL records are derived from structural PDB files.
(C) TrEMBL records are uncurated computational translations.
(D) TrEMBL records contain exclusively eukaryotic proteins.

2009

**171.** The Protein Data Bank (PDB) accepts three-dimensional structural data generated by which primary experimental techniques?
(A) X-ray crystallography, NMR spectroscopy, and Cryo-Electron Microscopy
(B) Mass spectrometry and High-performance liquid chromatography
(C) Sanger sequencing and Microarrays
(D) Western blotting and PCR

2021

**172.** Ensembl relies heavily on automated annotation pipelines. Which organismal genomes does Ensembl predominantly focus on?
(A) Vertebrates and selected model organisms
(B) Viruses and viroids
(C) Archaea exclusively
(D) Pathogenic bacteria

2006

**173.** Which boolean logic operator in Entrez expands a search by retrieving records containing either one or both of the specified terms?
(A) AND
(B) NOT
(C) OR
(D) XOR

2016

**174.** In a FASTA file, what immediately follows the “>” character on the first line?
(A) A unique sequence identifier and optional description
(B) The exact nucleotide sequence
(C) The translation frame indicator
(D) The organism’s complete taxonomy

2008

**175.** Which software tool is broadly recognized for providing a comprehensive suite of protein analysis programs, including isoelectric point calculation and peptide mass fingerprinting?

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2004

**154.** Which computational metric is most commonly utilized to assess the statistical confidence of individual branches within a phylogenetic tree?
(A) E-value
(B) Bootstrapping
(C) Z-score
(D) Root Mean Square Deviation (RMSD)

2011

**155.** Identifying a gene purely based on intrinsic genomic signals such as open reading frames and CpG islands, without comparing it to known transcripts, is termed:
(A) Homology-based prediction
(B) Ab initio gene prediction
(C) Comparative genomics
(D) Synteny mapping

2004

**156.** AUGUSTUS relies on which mathematical architecture to improve gene prediction accuracy in eukaryotic genomes?
(A) Simple Boolean logic gates
(B) Linear regression matrices
(C) Global dynamic programming
(D) General Hidden Markov Models (GHMM)

2004

**157.** Which ADMET property dictates how a pharmaceutical compound is enzymatically altered within the liver?
(A) Metabolism
(B) Absorption
(C) Distribution
(D) Excretion

2023

**158.** A molecular descriptor that encodes the presence or absence of specific structural fragments within a molecule as a binary string is known as a:
(A) 3D coordinate field
(B) Molecular fingerprint
(C) Pharmacophore map
(D) Topographical map

2005

**159.** In pharmacophore modeling, which of the following is considered a standard pharmacophoric feature?
(A) Atomic mass
(B) Hydrogen bond donor
(C) Isotopic ratio
(D) Melting point

2010

**160.** Match the QSAR dimensionality in List I with its descriptor type in List II.
List I: (Dimensionality)
P. 1D QSAR
Q. 2D QSAR
R. 3D QSAR
S. 4D QSAR
List II: (Descriptor Type)
1. Incorporates conformational ensembles and multiple structural states
2. Uses global properties like molecular weight and pKa
3. Employs topological indices and connectivity matrices
4. Analyzes spatial interaction fields (e.g., CoMFA)
(A) P-2, Q-3, R-4, S-1
(B) P-1, Q-2, R-3, S-4
(C) P-3, Q-1, R-4, S-2
(D) P-4, Q-3, R-2, S-1

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2008

**161.** Which molecular docking search algorithm sequentially places molecular fragments into the binding site and dynamically links them?
(A) Simulated Annealing
(B) Monte Carlo
(C) Incremental Construction
(D) Genetic Algorithm

2008

**162.** Reverse vaccinology leverages whole genome sequencing to predict vaccine candidates. Who pioneered this approach by applying it to Neisseria meningitidis?
(A) Craig Venter
(B) Rino Rappuoli
(C) Margaret Dayhoff
(D) David Lipman

2021

**163.** B-cell epitopes are typically identified by plotting sequence propensity scales. Which physicochemical property is most strongly correlated with antigenic regions?
(A) Hydrophobicity
(B) Aromaticity
(C) Isoelectric point
(D) Hydrophilicity

2017

**164.** Support Vector Machines (SVM) classify biological data by mapping inputs into high-dimensional feature spaces using which mathematical technique?

(A) De novo structure-based drug design
(B) Pharmacophore searching
(C) 2D QSAR
(D) Reverse vaccinology

2003
**187.** In molecular docking, AutoDock Vina is renowned for significantly improving which operational parameter over older AutoDock versions?
(A) High-resolution visual rendering
(B) Computational speed and multi-threading capabilities
(C) Covalent bond breaking simulations
(D) Whole-genome screening capabilities

2009
**188.** In QSAR modeling, topological indices derived directly from the connection table of atoms and bonds (e.g., Wiener index) represent which dimension of descriptors?
(A) 2D
(B) 1D
(C) 3D
(D) 4D

2008
**189.** A key advantage of in silico immunoinformatics over traditional laboratory screening is:
(A) The rapid, cost-effective triaging of thousands of potential epitopes
(B) The absolute guarantee of clinical efficacy
(C) The complete elimination of experimental animal testing
(D) The ability to synthesize antibodies computationally

2015
**190.** MHC Class I molecules predominantly present endogenous peptide fragments to which specific type of immune cell?
(A) $CD_8$+ Cytotoxic T-cells
(B) $CD_4$+ Helper T-cells
(C) B-lymphocytes
(D) Natural Killer cells

2004
**191.** Artificial Neural Networks employ which mathematical algorithm to systematically minimize classification error during the training phase?
(A) Monte Carlo simulations
(B) Backpropagation
(C) Neighbor-Joining
(D) Needleman-Wunsch

2018
**192.** Which machine learning algorithm classifies a data point based on the majority vote of the '$k$' most similar instances in the feature space?
(A) K-Nearest Neighbors
(B) Support Vector Machine
(C) Decision Tree
(D) Principal Component Analysis

2002
**193.** In predictive disease modeling in animal health, 'Omics' data integration provides a holistic view. Which omics tier directly studies the complete set of small-molecule intermediates and products?
(A) Metabolomics
(B) Genomics
(C) Transcriptomics
(D) Proteomics

2014
**194.** The specific region of a tRNA molecule that physically base-pairs with mRNA during translation is termed the:
(A) D-loop
(B) Acceptor stem
(C) Anticodon loop
(D) TΨC loop

2006
**195.** A database that meticulously curates sequence variations and correlates them strictly with inherited human diseases is best classified as:
(A) A boutique or specialized database (e.g., OMIM)
(B) A primary generalized database
(C) A composite genomic browser
(D) A secondary protein domain database

2018
**196.** The fundamental agreement binding the INSDC consortium requires that:
(A) Nucleotide sequence submissions to any member are automatically mirrored to the others daily
(B) All databases must utilize the exact same flat-file formatting
(C) Protein structural models must accompany all DNA sequences
(D) Members charge uniform fees for sequence downloads

2005
**197.** Which UniProt division consists of automatically annotated records waiting for manual review?
(A) Swiss-Prot
(B) TrEMBL

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process most directly benefits from the massive influx of AlphaFold-predicted structures?
(A) Structure-based virtual screening and molecular docking
(B) 1D QSAR
(C) Microarray expression analysis
(D) PCR primer design

2025
**280.** Match the bioinformatics sequence formats in List I with their characteristic start symbols in List II.
List I: (Sequence Format)
P. FASTA
Q. FASTQ
R. GenBank
S. EMBL
List II: (Start Symbol)
1. @
2. >
3. ID
4. LOCUS
(A) P-2, Q-1, R-4, S-3
(B) P-1, Q-2, R-3, S-4
(C) P-3, Q-4, R-2, S-1
(D) P-2, Q-1, R-4, S-3

2023
**281.** Genomic selection in animal health utilizes genome-wide marker data to calculate which specific quantitative metric?
(A) Estimated Breeding Value (EBV)
(B) Absolute Transcriptomic Yield (ATY)
(C) Phylogenetic Bootstrapping Score (PBS)
(D) Log-Odds Substitution Rate

2006
**282.** The structural arrangement of distinct protein subunits into a multi-subunit functional complex defines which level of protein structure?
(A) Quaternary
(B) Primary
(C) Secondary
(D) Tertiary

2025
**283.** The primary role of the dbSNP database maintained by NCBI is to catalog:
(A) Short nucleotide variations, primarily Single Nucleotide Polymorphisms
(B) Full viral genomes
(C) 3D protein coordinate grids
(D) Known metabolic pathways

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2023
**284.** Which biological resource represents a massive, internationally collaborative secondary database of protein families, domains, and functionally important sites?
(A) InterPro
(B) dbEST
(C) PDB
(D) PubMed

2008
**285.** In the context of UniProt, what ensures that Swiss-Prot data remains non-redundant despite multiple researchers sequencing the identical protein?
(A) Expert curators manually merge reports of the identical protein into a single, comprehensive record
(B) Automated algorithms instantly delete any sequence with >90% similarity
(C) Submissions are restricted to one per species per year
(D) The database relies exclusively on X-ray crystallography confirmation

2025
**286.** SCOP classification defines a 'Fold' by evaluating whether distinct proteins share identical:
(A) Major secondary structures in the same topological arrangement
(B) Evolutionary ancestors
(C) Biological functions
(D) Genomic locations

2006
**287.** Which feature of the Ensembl Genome Browser uniquely aids in evolutionary genomics across distinct vertebrate species?
(A) Integrated comparative genomics tracks, displaying synteny and ortholog alignments
(B) Standalone offline accessibility
(C) Real-time simulation of protein docking
(D) Experimental synthesis of novel gene arrays

2009
**288.** The sequence processing tool ORF Finder typically identifies an Open Reading Frame as a sequence bounded by:
(A) An ATG start codon and an in-frame stop codon, exceeding a minimal length threshold
(B) Two identical restriction enzyme sites
(C) A promoter and an enhancer region
(D) The 5-prime cap and the poly-A tail

(D) Outgroup

2021

**208.** Which distance-based phylogenetic algorithm explicitly constructs an unrooted tree and relaxes the strict molecular clock assumption?
(A) Neighbor-Joining
(B) Maximum Parsimony
(C) UPGMA
(D) Maximum Likelihood

2003

**209.** Long-branch attraction is a statistical artifact predominantly affecting which phylogenetic method?
(A) Neighbor-Joining
(B) Maximum Parsimony
(C) UPGMA
(D) Bootstrapping

2018

**210.** Arrange the progressive sequence of logical steps used by an ab initio gene prediction algorithm (e.g., GENSCAN):
I. Splice site prediction (donor/acceptor sites)
II. Open Reading Frame (ORF) identification
III. Start and stop codon localization
IV. Exon assembly into a gene model
Which of the following is the correct sequence?
(A) II, III, I, IV
(B) III, I, II, IV
(C) III, II, I, IV
(D) I, III, II, IV

2019

**211.** Lipinski's Rule of Five predicts poor absorption if a molecule contains more than how many hydrogen bond acceptors (sum of N and O atoms)?
(A) 2
(B) 5
(C) 10
(D) 15

2015

**212.** In Computer-Aided Drug Design, which methodology maps a theoretical grid around aligned ligand molecules to compute steric and electrostatic interaction fields?
(A) 3D-QSAR (e.g., CoMFA)
(B) Homology modeling
(C) Support Vector Machines
(D) Flexible molecular docking

2001

**213.** Molecular docking scoring functions that rely fundamentally on basic physical principles such as Coulombic and Lennard-Jones potentials are categorized as:
(A) Knowledge-based scoring functions
(B) Empirical scoring functions
(C) Force-field-based scoring functions
(D) Pharmacophore models

2022

**214.** Which specific descriptor type in QSAR directly addresses the three-dimensional volumetric shape of a drug molecule?
(A) topological index
(B) Steric parameter
(C) Electronic parameter
(D) Constitutional descriptor

2013

**215.** Reverse vaccinology aims to identify candidate protective antigens by computationally analyzing:
(A) Complete genomic sequences of the target pathogen
(B) Transcriptomic profiles of the host organism
(C) Crystal structures of established vaccine adjuvants
(D) The metabolomic output of infected tissues

2015

**216.** T-cell epitopes are generally how long in amino acid sequence when presented by MHC Class I molecules?
(A) 3-5 residues
(B) 8-11 residues
(C) 15-24 residues
(D) >30 residues

2024

**217.** Which term describes the capability of a Deep Learning model to automatically identify relevant features from raw data (e.g., sequence) without manual human curation?
(A) Feature extraction / Representation learning
(B) Feature engineering
(C) Cross-validation
(D) Linear regression

NODIA

2025

**218.** AlphaFold utilizes multiple sequence alignments (MSAs) to infer which structural constraints before constructing a 3D model?
(A) Co-evolutionary distance maps between residue pairs
(B) Intron-exon boundary probabilities

2022

**239.** Which molecular property evaluates a molecule's lipophilicity, crucial for penetrating lipid bilayers as per Lipinski's Rule of Five?
(A) $\log P$
(B) Polar surface area
(C) Molecular weight
(D) pKa

2004

**240.** Evaluate the following statements on molecular descriptors:
I. 1D descriptors include molecular weight.
II. 2D descriptors include connectivity indices.
III. 3D descriptors are strictly independent of molecular conformation.
IV. 3D descriptors evaluate spatial fields.
Which of the above statement(s) is/are correct?
(A) I, II and IV
(B) I, II and III
(C) II, III and IV
(D) I, III and IV

2011

**241.** Pharmacophore modeling relies heavily on identifying the spatial arrangement of:
(A) Functional groups necessary for receptor recognition
(B) The target protein's entire backbone
(C) The full nucleotide sequence of the pathogen
(D) Every single water molecule in the binding pocket

2011

**242.** In Computer-Aided Drug Design, virtual screening leverages docking algorithms primarily to:
(A) Rapidly evaluate and rank a massive library of compounds against a structural target
(B) Simulate long-term molecular dynamics of a single protein over microseconds
(C) Synthesize chemical compounds iteratively
(D) Edit the genome to remove susceptible alleles

2012

**243.** Partial Least Squares (PLS) regression is extensively utilized in which modeling approach to handle highly correlated 3D descriptors?
(A) 3D-QSAR (e.g., CoMFA)
(B) Ab initio gene prediction
(C) Random Forest classification
(D) Multiple sequence alignment

2004

**244.** B-cell epitopes located on the surface of a pathogenic protein must be physically accessible to antibodies. Which predictive scale is critical for assessing this accessibility?
(A) Hydropathy / Hydrophilicity
(B) Isoelectric focusing
(C) Molecular mass
(D) HalF-life degradation

2022

**245.** Reverse vaccinology dramatically reduces the time required for vaccine discovery primarily by bypassing:
(A) The cultivation of dangerous, fastidious pathogens in laboratory environments
(B) The need for complete pathogen genome sequencing
(C) Phase III clinical trials in human populations
(D) The reliance on computing clusters

2013

**246.** Artificial Neural Networks model synaptic weights, which computationally represent:
(A) The strength or importance of connections between node layers
(B) The physical mass of the algorithm
(C) The number of hidden layers
(D) The learning rate parameter

2009

**247.** Support Vector Machines utilize specific functions (like linear, polynomial, or radial basis) to handle non-linear data classifications. These functions are generically termed:
(A) Kernels
(B) Matrices
(C) Hyperparameters
(D) Nodes

2022

**248.** In molecular dynamics simulations, the set of mathematical functions and parameters used to describe the potential energy of a system is termed a:
(A) Force field
(B) Quantum kernel
(C) Neural net
(D) Substitution matrix

2008

**249.** Precision livestock farming integrates real-time sensor data (like accelerometers) with genomic data. The primary analytical tool applied to interpret these massive, complex datasets is:
(A) Machine learning and Artificial Intelligence algorithms
(B) Needleman-Wunsch dynamic programming
(C) Basic 1D QSAR

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(C) An ambiguous amino acid
(D) An intron boundary

2014
**229.** The BioEdit software package integrates a local implementation of which powerful alignment algorithm for multiple sequence alignment?
(A) ClustalW
(B) Needleman-Wunsch
(C) DALI
(D) AutoDock

2019
**230.** Evaluate the following statements regarding BLAST variants:
I. blastn compares nucleotide query against nucleotide database.
II. blastp compares protein query against protein database.
III. tblastn compares protein query against translated nucleotide database.
Which of the above statement(s) is/are correct?
(A) I, II and III
(B) I and II
(C) I and III
(D) II and III

2002
**231.** Which parameter dictates the minimum score required for a sequence match to be reported by a heuristic search algorithm?
(A) The $E$-value threshold
(B) The gap extension penalty
(C) The transition probability
(D) The substitution rate

2001
**232.** An affine gap penalty mathematically addresses the biological observation that:
(A) A single contiguous multi-residue insertion is more probable than multiple independent single-residue insertions
(B) Mutations occur more frequently than insertions
(C) Purines mutate to pyrimidines at a constant rate
(D) Protein folding domains are highly conserved

2007
**233.** The Smith-Waterman algorithm identifies local alignments by utilizing a dynamic programming matrix where negative cell scores are:
(A) Replaced with zero
(B) Multiplied by the gap penalty
(C) Converted to absolute positive values
(D) Averaged with adjacent cells

2012
**234.** Which multiple sequence alignment strategy strictly prevents iterative realignment of sequences once they have been grouped in the guide tree?
(A) Progressive method (e.g., ClustalW)
(B) Iterative method (e.g., MUSCLE)
(C) Consistency-based method (e.g., T-Coffee)
(D) Stochastic method (e.g., SAGA)

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2023
**235.** In phylogenetic trees, a node represents:
(A) A point of sequence mutation or speciation, implying an ancestor
(B) An extant operational taxonomic unit (OTU)
(C) The length of evolutionary time elapsed
(D) A continuous stretch of identical amino acids

2013
**236.** The Neighbor-Joining method constructs an unrooted phylogenetic tree. Which procedure must be computationally applied later to provide evolutionary directionality?
(A) Rooting the tree using an outgroup
(B) Bootstrapping the alignment
(C) Calculating Maximum Likelihood probabilities
(D) Converting the distance matrix to a character matrix

2024
**237.** Maximum Parsimony strictly evaluates informative sites. An informative site must contain at least how many distinct character states?
(A) Two, each present in at least two different taxa
(B) One
(C) Three, evenly distributed
(D) Four unique nucleotides

2021
**238.** Homology-based gene prediction significantly outperforms ab initio prediction in which specific scenario?
(A) Annotating highly conserved orthologous genes in closely related species
(B) Predicting completely novel genes with no known evolutionary homologs
(C) Identifying pseudogenes and non-coding RNAs in unsequenced domains
(D) Predicting the precise 3D fold of an unknown protein

(C) Ligand binding affinities
(D) Pharmacokinetic absorption rates

2010
**219.** The application of Omics data integration in precision livestock farming is fundamentally aimed at transitioning veterinary medicine from a reactive discipline to a:
(A) Predictive and preventive discipline
(B) Purely theoretical discipline
(C) Computationally obsolete discipline
(D) Purely structural discipline

2023
**220.** Match the AI/ML algorithms in List I with their typical bioinformatics applications in List II.
List I: (Algorithm)
P. Random Forest
Q. Hidden Markov Models
R. Support Vector Machines
S. Artificial Neural Networks
List II: (Application)
1. Sequence profile scanning and multiple alignment
2. High-dimensional omics classification via hyperplanes
3. Non-linear deep feature extraction (e.g., transcriptomics)
4. Robust ensemble classification of polygenic traits
(A) P-4, Q-1, R-2, S-3
(B) P-1, Q-2, R-3, S-4
(C) P-3, Q-4, R-1, S-2
(D) P-2, Q-3, R-4, S-1

2002
**221.** Which molecule serves as the direct physical intermediary linking nucleotide codons to specific amino acids during translation?
(A) Transfer RNA (tRNA)
(B) Ribosomal RNA (rRNA)
(C) Messenger RNA (mRNA)
(D) Small nuclear RNA (snRNA)

2024
**222.** The NCBI Entrez system natively cross-links records. A query for a gene sequence can directly lead to a 3D structure by linking which two databases?
(A) dbSNP to GEO
(B) GenBank to MMDB (NCBI's structural database)
(C) PubMed to OMIM
(D) SRA to TrEMBL

2006
**223.** What specific organizational format ensures the global synchronization of sequence records between GenBank, EMBL, and DDBJ?
(A) Nightly automated data exchange protocols
(B) Each database strictly shares a unified common SQL server
(C) Manual transcription of records by a central committee
(D) Exclusive reliance on PubMed citations

2023
**224.** Which structural database categorizes protein domains structurally by utilizing a fully automated computational algorithm, without manual intervention?
(A) FSSP (DALI database)
(B) SCOP
(C) CATH
(D) PDB

2003
**225.** A researcher wishing to submit an exceptionally long or complex annotated sequence, such as a full bacterial genome, to GenBank should use:
(A) Sequin / $tbl_2asn$
(B) BankIt
(C) BLAST
(D) BioEdit

2025
**226.** The UCSC Genome Browser provides distinct "tracks" that correspond to:
(A) Individual chromatograms from Sanger sequencers
(B) Superimposed layers of biological annotation aligned to a reference genome
(C) 3D stereoscopic views of protein structures
(D) Real-time tracking of livestock populations

2024
**227.** In the Sequence Retrieval System (SRS), the ability to search across disparate databases efficiently is governed by:
(A) Indexing the flat files while leaving the raw data in its native format
(B) Data warehousing (copying all databases into one format)
(C) Translating all sequences into binary formats
(D) Relying exclusively on BLAST heuristics

2006
**228.** In standard FASTA format, an asterisk () at the end of an amino acid sequence explicitly denotes:
(A) A stop codon
(B) A sequencing error

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(D) FASTA heuristic searches

2015

**250.** Evaluate the following statements regarding reverse vaccinology:

I. It requires whole genome sequences.
II. It identifies potential antigens mathematically rather than biochemically.
III. It can identify non-structural antigens that traditional vaccinology misses.
IV. It bypasses the need for ultimate in vivo testing.
V. It relies on algorithms predicting surface localization.

Which of the above statement(s) is/are correct?
(A) I, II, III and V
(B) I, II, IV and V
(C) II, III, IV and V
(D) I, III, IV and V

2024

**251.** Which mechanism enables a single eukaryotic gene to encode multiple distinct protein isoforms?
(A) Alternative splicing
(B) Reverse transcription
(C) Frameshifting
(D) Polyadenylation

2021

**252.** A researcher discovers a novel enzyme in an extreme thermophile. Which primary database should she immediately query to determine if the sequence is novel?
(A) Protein Data Bank (PDB)
(B) GenBank / nr (non-redundant database)
(C) Ensembl Genome Browser
(D) AnimalQTLdb

2022

**253.** The PIR-PSD (Protein Information Resource - Protein Sequence Database) distinguishes itself from TrEMBL by focusing on classifying proteins into:
(A) Highly curated evolutionary superfamilies
(B) 3D coordinate folds purely based on X-ray crystallography
(C) Untranslated RNA sequences
(D) Genomic contig assemblies

2002

**254.** CATH and SCOP utilize different methodologies for protein structural classification. Which core difference is fundamental?
(A) CATH is partially automated using structural algorithms; SCOP relies more heavily on expert manual curation
(B) SCOP is purely automated; CATH relies entirely on manual classification
(C) CATH only catalogs viral proteins; SCOP catalogs vertebrate proteins
(D) SCOP ignores evolutionary relationships; CATH relies solely on them

2005

**255.** In bioinformatics literature, RefSeq represents a database of computationally and manually curated sequences. Its primary advantage over GenBank's standard repository is:
(A) RefSeq provides a single, non-redundant, stable reference record for each biological molecule
(B) RefSeq contains every single sequenced read submitted, maximizing size
(C) RefSeq archives exclusively structural coordinates
(D) RefSeq deletes all records older than five years

2014

**256.** The AnimalQTLdb archives associations between genomic markers and observed phenotypes. This database is fundamentally structured to support which agricultural application?
(A) Marker-Assisted Selection (MAS) and genomic breeding
(B) De novo drug synthesis
(C) Viral capsid structural modeling
(D) RNA secondary structure prediction

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2004

**257.** In a boolean search within Entrez, constructing the query ($BRCA_1$ OR $BRCA_2$) AND human will definitively yield:
(A) Records containing either $BRCA_1$ or $BRCA_2$, strictly within humans
(B) Only records that contain both $BRCA_1$ and $BRCA_2$ in humans
(C) All human records excluding $BRCA_1$ and $BRCA_2$
(D) Records of $BRCA_1$ in humans, and $BRCA_2$ in all other organisms

2018

**258.** Which standard sequence format structurally relies on specific keyword tags at the start of each line, such as 'ID', 'AC', 'DE', and 'SQ'?
(A) EMBL
(B) FASTA
(C) GenBank
(D) PDB

2007

**259.** A key limitation of the basic FASTA format is its inability to natively support:
(A) Complex biological feature annotations (e.g., exon/intron boundaries) within the sequence file itself
(B) Protein sequences
(C) Sequences longer than 10,000 bases
(D) Multiple sequences in a single file

2012

**260.** Select the correct answer to the following question consisting of Assertion (A) and Reason (R):
**Assertion (A):** The Smith-Waterman algorithm is computationally more demanding than heuristic algorithms like BLAST.
**Reason (R):** Smith-Waterman explores the entire dynamic programming matrix to guarantee mathematical optimality, whereas BLAST restricts the search space using short word seeds.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2017

**261.** An $E$-value of $1 \times 10^{-50}$ in a BLAST result strongly implies that:
(A) The alignment is likely an artifact of a low-complexity region
(B) The match is biologically significant and evolutionary homology is highly probable
(C) There is a 50% chance the alignment is random
(D) The sequences share exactly 50 identical residues

2009

**262.** Which dynamic programming concept ensures that the alignment algorithm computationally remembers the path yielding the highest score to generate the final alignment string?
(A) Traceback
(B) Gap initialization
(C) Affine modeling
(D) Statistical normalization

2018

**263.** A BLOSUM matrix is mathematically constructed by computing log-odds ratios based on observed substitution frequencies. A positive score in a BLOSUM matrix indicates:
(A) The substitution occurs more frequently than expected by chance
(B) The substitution occurs less frequently than expected by chance
(C) The amino acids are completely structurally dissimilar
(D) A gap penalty must be applied

2014

**264.** Clustal Omega replaced ClustalW largely because it introduced which algorithm allowing it to handle hundreds of thousands of sequences efficiently?
(A) Hidden Markov Models (HMMs) via the mBed algorithm
(B) Standard dynamic programming
(C) Neural Networks
(D) Purely random seed matching

2025

**265.** Consistency-based multiple sequence alignment algorithms, such as T-Coffee, attempt to maximize agreement between a multiple alignment and a pre-computed library of:
(A) Pairwise alignments
(B) Phylogenetic trees
(C) 3D structures
(D) Sequence fragments

2008

**266.** A phylogram is explicitly distinguished from a cladogram because its branch lengths directly represent:
(A) The magnitude of evolutionary change or elapsed time
(B) The geographical distance between species
(C) The certainty (bootstrap value) of the node
(D) The total molecular weight of the analyzed sequences

2023

**267.** Which phenomenon introduces noise into phylogenetic analysis by creating identical character states in separate lineages not due to shared ancestry (e.g., convergent evolution)?
(A) Homoplasy
(B) Homology
(C) Orthology
(D) Synteny

2017

**268.** Maximum Likelihood phylogenetic analysis relies heavily on selecting an appropriate:
(A) Mathematical model of nucleotide substitution (e.g., General Time Reversible)
(B) Outgroup from a completely different kingdom

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(C) Three-dimensional alignment structural template
(D) Affine gap penalty value

2005
**269.** Eukaryotic gene prediction is substantially more complex than prokaryotic prediction primarily due to the presence of:
(A) Introns
(B) Stop codons
(C) Plasmids
(D) Operons

2011
**270.** Homology-based gene prediction is highly reliable because it leverages the evolutionary principle that:
(A) Coding sequences (exons) are generally more highly conserved than non-coding sequences (introns)
(B) Introns mutate at exactly the same rate as exons
(C) Intergenic regions define the final protein structure
(D) Splice sites are highly variable across conserved species

2005
**271.** According to Lipinski's Rule of Five, an orally active drug generally violates no more than how many of the specified criteria?
(A) One
(B) Zero
(C) Two
(D) Three

2012
**272.** In Computer-Aided Drug Design, a common technique to refine a docked pose and simulate real physiological interactions over time is:
(A) Molecular Dynamics (MD) simulations
(B) 1D QSAR
(C) Ab initio gene assembly
(D) Bootstrapping

2006
**273.** AutoDock utilizes a Lamarckian Genetic Algorithm. How does the 'Lamarckian' aspect modify a standard genetic algorithm?
(A) It incorporates a local conformational search whose improved pose is passed directly back to the genome
(B) It permanently prevents any random mutations during docking
(C) It deletes individuals from the population randomly
(D) It restricts docking strictly to rigid body assumptions

2023
**274.** In QSAR modeling, validating the model purely on the training set can lead to which significant statistical error?
(A) Overfitting
(B) Underfitting
(C) Linear dependence
(D) Dimensionality reduction

2011
**275.** Reverse vaccinology heavily relies on specific predictive algorithms to ensure the selected antigen target is localized where in the pathogen?
(A) Surface-exposed or secreted
(B) Buried deep within the structural core
(C) Bound tightly to genomic DNA
(D) Exclusively located in the mitochondria

2015
**276.** T-cell epitopes are generally inherently:
(A) Linear, because they are presented as digested peptides on MHC molecules
(B) Highly conformational and non-linear
(C) Composed of purely hydrophilic amino acids
(D) Independent of MHC binding capabilities

2023
**277.** The 'black box' problem in Artificial Neural Networks refers to the difficulty in:
(A) Understanding exactly how the network combines input features to reach its final classification
(B) Providing the network with sufficient training data
(C) Selecting the correct coding language for the algorithm
(D) Calculating the physical storage size of the output

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2012
**278.** Which unsupervised learning technique is primarily used to reduce the dimensionality of high-throughput omics data while retaining the most significant variance?
(A) Random Forest
(B) Principal Component Analysis (PCA)
(C) Linear Regression
(D) K-Means Clustering

2014
**279.** AlphaFold significantly accelerates structural bioinformatics. Which downstream CADD

(C) UniParc
(D) UniRef

2025
**198.** The CATH classification system employs the DALI algorithm extensively at which level to detect structural similarities?
(A) Class
(B) Architecture
(C) Topology
(D) Homologous superfamily

2016
**199.** The NCBI Genome database organizes high-throughput sequencing data primarily into which refined, well-annotated reference standard?
(A) RefSeq genomes
(B) SRA records
(C) dbEST sequences
(D) GEO profiles

2017
**200.** Select the correct answer to the following question consisting of Assertion (A) and Reason (R):
**Assertion (A):** Entrez uses inverted indices to accelerate complex Boolean queries across distinct databases.
**Reason (R):** Inverted indices map individual terms to the distinct documents (or records) containing them, bypassing the need to read every document during a query.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2013
**201.** Which metadata field in a GenBank flat file details the specific biological organism from which the sequence was derived, including taxonomic classification?
(A) LOCUS
(B) SOURCE
(C) FEATURES
(D) ORIGIN

2001
**202.** Evaluate the following statements regarding the ExPASy Translate tool:
I. Mitochondrial genomes utilize variants of the standard genetic code.
II. Eukaryotic translation completely ignores standard start codons.
III. Splice sites actively alter the translation frame dynamically.
IV. Bacterial genomes lack stop codons entirely.
Which of the above statement(s) is/are correct?
(A) I only
(B) I and III
(C) II and IV
(D) I, II, III and IV

2009
**203.** Which dynamic programming parameter prevents the accumulation of biologically unrealistic, continuous gaps during sequence alignment?
(A) Substitution matrices
(B) Guide trees
(C) Affine gap penalties
(D) High $E$-value thresholds

2025
**204.** In the derivation of the BLOSUM matrices, a higher matrix number (e.g., BLOSUM80 vs BLOSUM45) implies that the matrix is tailored for comparing sequences that are:
(A) Highly divergent
(B) Closely related
(C) Structurally unrelated
(D) Evolutionarily distant

2023
**205.** The FASTA heuristic database search tool accelerates alignments by initially locating identical:
(A) 3D coordinate folds
(B) Motif profiles
(C) $K$-tuples
(D) Splice junctions

2009
**206.** Iterative multiple sequence alignment methods (e.g., MUSCLE) improve upon progressive methods primarily by:
(A) Re-evaluating and correcting errors made during initial pairwise alignments
(B) Eliminating the need for a scoring matrix entirely
(C) Restricting alignments to only three sequences simultaneously
(D) Operating exclusively on DNA sequences

2002
**207.** A monophyletic group on a phylogenetic tree is completely synonymous with which taxonomic term?
(A) Clade
(B) Grade
(C) Taxon

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2004

**289.** In dynamic programming sequence alignments, the trace-back step always originates from which cell in a global alignment matrix?
(A) The extreme bottom-right cell of the matrix
(B) The cell with the absolute highest score anywhere in the matrix
(C) The extreme top-left cell of the matrix
(D) Any cell containing a zero

2003

**290.** The BLAST heuristic algorithm relies on 'neighborhood words'. If a query word scores above a specific threshold '$T$' against a database word, what is triggered?
(A) An un-gapped local extension evaluates if a high-scoring segment pair (HSP) exists
(B) The search is immediately terminated
(C) A gap is permanently inserted
(D) A phylogenetic tree is automatically generated

2007

**291.** In a BLAST search, the primary purpose of 'masking' low-complexity regions (e.g., simple repeats) is to:
(A) Speed up the CPU time by 90%
(B) Prevent statistically significant but biologically spurious hits
(C) Increase the $E$-value of true homologs
(D) Force the algorithm to use dynamic programming

2025

**292.** In multiple sequence alignment, a 'profile' represents a probabilistic model of an aligned block. Aligning two profiles together is mathematically similar to:
(A) Aligning two distinct dynamic programming matrices
(B) Converting DNA directly to protein
(C) Rigid body molecular docking
(D) Randomizing phylogenetic branches

2025

**293.** Which term specifies distinct, non-homologous proteins that have evolved entirely independently to possess the exact same biological function?
(A) Analogs
(B) Orthologs
(C) Paralogs
(D) Xenologs

2012

**294.** Maximum Parsimony is generally considered least reliable when analyzing sequences that are:
(A) Highly divergent, accumulating numerous multiple hits at identical sites
(B) Extremely closely related
(C) Short in length
(D) Completely free of gap insertions

2005

**295.** Which technique evaluates the robustness of a phylogenetic tree by randomly resampling columns from the original sequence alignment to create pseudo-replicate datasets?
(A) Bootstrapping
(B) Jackknifing
(C) Cross-validation
(D) Hidden Markov Modeling

2001

**296.** Eukaryotic ab initio gene prediction algorithms primarily struggle with identifying which structural features accurately?
(A) Short initial and terminal exons bounded by massive introns
(B) Exact transcription start sites
(C) Unambiguous stop codons
(D) Standard poly-A signals

2006

**297.** In CADD, a basic ADMET prediction algorithm flags a compound with a molecular weight of 750 Da, 12 hydrogen bond donors, and a $\log P$ of 8. What is the most appropriate conclusion?
(A) The compound severely violates Lipinski's Rule of Five and is highly unlikely to be orally bioavailable
(B) The compound is an ideal candidate for an oral pharmaceutical
(C) The compound is definitely a potent broad-spectrum antibiotic
(D) The compound is highly hydrophilic and requires lipid encapsulation

2005

**298.** In molecular docking, a purely rigid search space requires evaluating a maximum of how many degrees of freedom (translational and rotational)?
(A) Three
(B) Six
(C) Nine
(D) Infinite

2009

**299.** Reverse vaccinology frequently screens bacterial outer membrane proteins (OMPs). Why are OMPs exceptional candidate antigens?
(A) They are highly surface-exposed, readily accessible to host antibodies

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determined through nuclear magnetic resonance spectroscopy and cryo-electron microscopy. Every entry receives a unique four-character alphanumeric code to facilitate identification and retrieval.

**10.** (B) P-2, Q-4, R-3, S-1
Structural biology relies on different classification systems to organize protein data. The Protein Data Bank serves as the primary archive of atomic coordinates. SCOP provides manual classification based on evolutionary relationships, while CATH uses a semi-automated approach considering class, architecture, and topology. FSSP utilizes purely automated structure comparisons through the DALI algorithm.

**11.** (A) UCSC Genome Browser
The UCSC Genome Browser is a widely used bioinformatics tool characterized by its intuitive track-based visual interface. It integrates various genomic annotations and utilizes the BLAT algorithm for rapid sequence alignment against reference genomes. This allows researchers to quickly visualize genes, regulatory elements, and comparative genomics data across multiple species within a single graphical framework.

**12.** (C) Quantitative trait loci and phenotypic traits
AnimalQTLdb is a specialized database designed to curate and map genetic markers associated with quantitative trait loci and phenotypic traits in livestock species. It serves as a comprehensive resource for animal scientists to link specific genomic regions to economically important characteristics like milk production, growth rate, and disease resistance, facilitating marker-assisted selection programs.

**13.** (D) p53 AND human NOT cancer
In the Entrez retrieval system, Boolean operators are used to refine search results. Combining terms with AND ensures all specified keywords are present, while the NOT operator excludes records containing unwanted terms. Therefore, using the query string including p53 and human while excluding cancer effectively retrieves specific genetic records relevant to healthy human cellular processes.

**14.** (C) Sequence Retrieval System (SRS)
The Sequence Retrieval System was a prominent bioinformatics tool developed to link heterogeneous flat-file databases using defined indices. Before the widespread dominance of the Entrez system, SRS allowed researchers to perform complex queries across multiple biological resources simultaneously. It played a crucial role in data integration by providing a unified interface for disparate sequence repositories.

**15.** (B) >
The FASTA file format is a standard text-based representation for nucleotide or peptide sequences. It requires a specific greater-than symbol to indicate the start of the description or header line. This convention allows bioinformatics software to distinguish between metadata and the actual raw sequence data, which follows on the subsequent lines of the file.

**16.** (D) //
In the GenBank flat-file format, a specific double-slash symbol string is used to mark the conclusion of a complete sequence record. This terminator allows parsing software to identify where one entry ends and the next begins within a multi-record file. It ensures the structural integrity of the database by providing a clear boundary for each entry.

**17.** (A) Local sequence alignment and manual editing of multiple alignments
BioEdit is a popular biological sequence alignment editor designed for use on Windows platforms. It provides a user-friendly interface for performing local sequence alignments and manually refining multiple sequence alignments. Bioinformaticians use it to manipulate sequences, visualize conserved regions, and prepare data for further phylogenetic analysis or structural modeling within a consolidated software environment.

**18.** (C) Six
The ExPASy Translate tool evaluates all six potential open reading frames for any given double-stranded DNA sequence. This includes three forward reading frames on the sense strand and three reverse reading frames on the complementary antisense strand. Analyzing all six frames is essential to identify the correct protein-coding region, especially when the gene orientation is unknown.

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**19.** (A) Optimal global alignment
The Needleman-Wunsch algorithm utilizes dynamic programming to determine the

# ANSWERS

**1.** (A) Reverse transcription
Reverse transcription is the biological process where genetic information flows from RNA back into DNA, catalyzed by the enzyme reverse transcriptase. This pathway is a critical step in the life cycle of retroviruses, such as HIV, allowing them to integrate their viral genetic material into the host cell's genome for replication.

**2.** (C) Hydroxyl group
The fundamental chemical distinction between ribose in RNA and deoxyribose in DNA lies at the 2-prime carbon atom of the pentose sugar. Ribose contains a hydroxyl group at this position, whereas deoxyribose has a hydrogen atom instead. This single oxygen difference significantly impacts the structural stability and biochemical reactivity of nucleic acids.

**3.** (A) OWL
Composite databases like OWL function by aggregating and integrating information from multiple primary biological data sources into a single, unified searchable resource. These systems do not store original submissions directly but rather provide a consolidated view, enhancing data accessibility and cross-referencing capabilities for researchers seeking comprehensive biological information from disparate origins.

**4.** (D) It stores computationally derived consensus patterns, motifs, and profiles
Secondary databases are specialized bioinformatic resources that store processed information derived from primary sequence data. These repositories focus on identifying and archiving computationally determined consensus patterns, motifs, and profiles. By highlighting conserved functional or structural domains within protein families, they provide deep biological insights that are not immediately apparent from raw sequences.

**5.** (B) EMBL, DDBJ, and GenBank
The International Nucleotide Sequence Database Collaboration consists of three major global repositories: EMBL in Europe, DDBJ in Japan, and GenBank in the United States. These organizations maintain a formal agreement to synchronize their data daily, ensuring that all new nucleotide sequence submissions are shared globally and remain freely accessible to the entire scientific community.

**6.** (C) Webin
Researchers submitting new or annotated nucleotide sequences to the European Molecular Biology Laboratory repository typically utilize the Webin tool. This web-based interface facilitates the entry of sequence data along with essential biological metadata and annotations. Proper submission ensures the sequence is assigned an accession number and integrated into the global sequence database network.

**7.** (D) Swiss-Prot
Swiss-Prot is the section of the UniProt Knowledgebase that contains protein sequence records characterized by high-quality, manual annotation. Expert curators extract biological information from peer-reviewed literature to ensure accuracy and minimize redundancy. This process provides researchers with reliable data concerning protein function, post-translational modifications, and domain structures across a wide variety of organisms.

**8.** (A) Identifying homologous proteins based on evolutionary superfamilies
The Protein Information Resource was established to assist bioinformaticians in identifying homologous proteins and organizing them into evolutionary superfamilies. By focusing on the functional and evolutionary relationships between sequences, PIR provides a systematic framework for understanding protein diversity. This classification helps in predicting the function of newly discovered proteins based on their evolutionary heritage.

**9.** (B) It accepts structural data generated exclusively by X-ray crystallography
The Protein Data Bank serves as the primary global archive for the three-dimensional structures of large biological molecules. While X-ray crystallography is a major source of data, the repository also accepts structures

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highlight predicted start and stop codons, aiding researchers in identifying valid open reading frames. However, it does not generate structural coordinates.

**65.** (C) Francis Crick
The foundational concept of the Central Dogma of molecular biology was initially proposed by Francis Crick in 1958. It describes the unidirectional flow of genetic information from DNA to RNA and then to protein. This framework provided a theoretical basis for understanding how genetic instructions are translated into functional molecules, serving as the cornerstone for modern molecular biology and genetics.

**66.** (B) It archives original experimental data submitted directly by researchers
A primary biological database is distinguished by its operational policy of archiving original experimental data submitted directly by researchers. These repositories, such as GenBank and EMBL, act as the definitive records for newly discovered sequences and structures. They maintain the data in its original form along with essential metadata, ensuring that the primary evidence remains available for the scientific community.

**67.** (B) Wellcome Trust Sanger Institute
The Ensembl Genome Browser is a collaborative project jointly maintained by the European Bioinformatics Institute and the Wellcome Trust Sanger Institute. It provides a centralized resource for the annotation and visualization of vertebrate genomes. By integrating various biological datasets and providing robust automated annotation pipelines, Ensembl supports genomic research across a wide range of model organisms and economically important species.

**68.** (B) Boolean logic applied to pre-computed inverted indices
The Entrez retrieval system utilizes a sophisticated computational strategy based on Boolean logic applied to pre-computed inverted indices. These indices map specific terms to the records containing them, allowing for rapid retrieval across multiple linked databases. By avoiding the need to scan entire records in real-time, Entrez provides a fast and efficient interface for accessing vast amounts of biological information.

**69.** (A) Both A and R are true and R is the correct explanation of A.
The EMBL flat-file format utilizes two-letter line codes, such as ID for identification and AC for accession numbers, to denote specific data fields. This standardized coding system enables bioinformatics parsers to rapidly extract specific metadata without the need to read the entire file. This efficiency is critical for high-throughput data processing and integration across different biological databases and tools.

**70.** (A) P-4, Q-3, R-1, S-2
Various bioinformatics tools employ distinct methodologies for sequence analysis. Clustal Omega uses guide trees for scalable progressive multiple alignment. MUSCLE utilizes an iterative approach with log-expectation scores. BLAST relies on heuristic pairwise alignment via $k$-mer seeding for speed. HMMER employs profile hidden Markov models for sensitive sequence searches. These specialized methods allow researchers to select the most appropriate tool.

**71.** (A) Opening a new gap
In pairwise sequence alignment, an affine gap penalty model distinguishes between the cost of starting a gap and extending one. A higher penalty is applied to opening a new gap compared to extending an existing one. This reflects the biological reality that a single mutational event often results in a multi-residue indel, making a single long gap more probable than multiple short ones.

**72.** (C) tfasta
The tfasta program is a specialized variant of the FASTA algorithm designed to search a translated DNA sequence database using a protein query. It achieves this by translating every DNA sequence in the database in all six potential reading frames and then comparing the protein query against these translations. This tool is effective for finding protein-coding regions within unannotated genomic data.

**73.** (A) Early gap insertion errors are not removed in later stages.
The 'once a gap, always a gap' rule is a defining characteristic of standard progressive multiple

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mathematically optimal global alignment between two sequences. By considering the entire length of both sequences, it seeks the highest overall similarity score based on a defined substitution matrix and gap penalties. This method is particularly effective for comparing sequences of similar length and high overall similarity.

**20.** (A) Both A and R are true and R is the correct explanation of A.
The Smith-Waterman algorithm is the preferred choice for identifying highly conserved local domains within divergent sequences. By initializing negative values in the scoring matrix to zero, the algorithm prevents poorly matching flanking regions from lowering the overall score. This allows for the detection of significant local similarities that a global alignment method might overlook or obscure.

**21.** (B) PAM250
The PAM250 substitution matrix is derived from a mathematical model that extrapolates the probability of amino acid substitutions over significant evolutionary time. It represents a distance corresponding to 250 accepted point mutations per 100 residues. This matrix is specifically designed to facilitate the alignment of distantly related protein sequences by reflecting long-term evolutionary divergence patterns.

**22.** (C) Biological indels usually occur as single events rather than independent mutations.
Affine gap penalty models utilize different values for opening and extending a gap because biological insertion or deletion events typically occur as single mutations involving multiple residues. Setting a lower penalty for gap extension reflects the observation that extending an existing gap is more evolutionarily probable than creating a new one, resulting in more biologically realistic sequence alignments.

**23.** (D) Pure random chance
The Expect value in a BLAST search represents the number of distinct sequence alignments that are expected to occur by pure random chance within a database of a specific size. A lower E-value indicates a higher statistical significance, suggesting that the observed match is likely due to biological homology rather than a coincidental similarity between random sequences.

**24.** (A) blastx
The blastx program is a specific variant of the BLAST algorithm designed to search a protein database using a nucleotide query. It accomplishes this by translating the DNA sequence into all six potential reading frames and comparing each translation against the protein records. This tool is invaluable for identifying coding regions in newly sequenced genomic DNA.

**25.** (B) Initial alignment errors made at early branching nodes cannot be corrected later
A primary disadvantage of the progressive multiple sequence alignment approach used by ClustalW is its sensitivity to early errors. Once sequences are aligned at the initial branching nodes of the guide tree, those alignments are fixed. Any mistakes made during these early stages cannot be corrected later, which can lead to suboptimal final results in divergent datasets.

**26.** (B) A library of pairwise global and local alignments
T-Coffee improves the accuracy of multiple sequence alignments by incorporating a comprehensive library of both global and local pairwise alignments. Unlike standard progressive methods that rely on a single scoring matrix, T-Coffee uses this pre-computed information to build a consensus. This mathematical framework helps reduce the impact of early errors and produces more robust alignments for divergent sequences.

**27.** (A) Orthologs
Orthologs are defined as homologous genes in different species that originated from a single gene in a common ancestor via a speciation event. These genes typically retain the same biological function across different organisms. Identifying orthologous relationships is fundamental for comparative genomics, functional annotation, and reconstructing the evolutionary history of diverse biological lineages.

**28.** (C) Branch lengths in a cladogram do not represent evolutionary time or amount of character change
Cladograms and phylograms are both used to represent evolutionary relationships, but they differ in how branch lengths are interpreted. In a cladogram, the branch lengths are arbitrary and do not represent evolutionary time or the amount of character change. In contrast, a phylogram

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(B) They are completely immune to evolutionary mutation
(C) They are identical across all domains of life
(D) They cannot be presented by MHC molecules

2009

**300.** In veterinary bioinformatics, integrating high-throughput genomic marker data with Machine Learning (e.g., Random Forests) fundamentally shifts breeding from purely phenotypic evaluation to:
(A) Direct CRISPR-mediated silencing of deleterious alleles.
(B) Complete reliance on transcriptomic cloning techniques.
(C) Highly accurate, predictive genomic selection of traits.
(D) Abolishing artificial selection in breeding populations.

**********

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model is to establish a mathematical correlation between the structural descriptors of chemical compounds and their observed biological activities. By identifying which molecular properties influence potency or toxicity, QSAR models enable researchers to predict the performance of new compounds and optimize their structures during the drug discovery process.

**39.** (A) Both A and R are true and R is the correct explanation of A.
Cross-validation procedures, such as the leave-one-out method, are essential in the development of 3D QSAR models. These statistical tests assess the predictive capability of the model by ensuring it can accurately forecast the activity of molecules not included in the training set. This prevents the model from simply memorizing data, thereby ensuring its robustness and scientific validity.

**40.** (A) I and III
B-cell epitopes are classified as either linear or conformational. Linear epitopes consist of a continuous sequence of amino acids, and their prediction often utilizes hydrophilicity scales to identify surface-exposed regions. In contrast, conformational epitopes depend on the three-dimensional folding of the protein. Most natural epitopes are conformational, involving residues that are distant in the primary sequence but spatially adjacent.

**41.** (B) Whole genomic sequence data analyzed in silico
Reverse vaccinology represents a modern approach to vaccine development that begins with the computational analysis of a pathogen's complete genomic sequence. By using bioinformatics tools to screen for potential antigens in silico, researchers can identify candidates that might be difficult to discover through traditional biochemical methods. This accelerates the process of finding protective antigens for vaccine formulation.

**42.** (B) T-cell epitopes
Major Histocompatibility Complex binding prediction matrices are essential tools in immunoinformatics for identifying potential T-cell epitopes. These algorithms estimate the affinity with which short peptide fragments from a pathogen will bind to MHC or HLA molecules. Efficient binding is a prerequisite for peptide presentation to T-cells, making these predictions vital for designing subunit vaccines and understanding cellular immunity.

**43.** (D) Support Vector Machine
A Support Vector Machine is a powerful machine learning algorithm used for classification and regression tasks. It operates by establishing an optimal hyperplane that maximizes the marginal distance between different categories of data points in a high-dimensional feature space. This mathematical approach ensures robust performance and effective generalization, particularly when dealing with complex biological datasets like gene expression.

**44.** (A) IV, I, III, II
The AlphaFold deep learning architecture predicts protein structures through a series of logical steps. The process begins with input sequence processing, followed by the generation of a multiple sequence alignment. Next, feature extraction is performed using sophisticated transformer neural networks (Evoformer). Finally, the system generates the 3D spatial coordinates, resulting in a high-accuracy structural model of the protein.

**45.** (B) Identifying biomarker combinations for trait selection.
In precision livestock farming, Random Forest algorithms are frequently applied to large-scale omics data to identify complex biomarker combinations for trait selection. As an ensemble learning method, it builds multiple decision trees to handle the high dimensionality and non-linear relationships characteristic of genomic and transcriptomic datasets. This helps breeders select animals with superior phenotypic traits more accurately.

**46.** (B) Cloverleaf
Transfer RNA molecules typically fold into a characteristic two-dimensional cloverleaf shape. This structure is maintained by base-pairing between different regions of the single-stranded RNA, resulting in four main arms: the acceptor stem, the D-loop, the anticodon loop, and the T-psi-C loop. This specific arrangement is crucial for its function as an adapter during the translation of genetic information.

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**47.** (C) Specialized/Boutique database
Specialized or boutique databases are bioinformatic resources that compile domain-specific information tailored to particular research areas, such as metabolic pathways, disease mutations, or specific organismal groups. Unlike generalized archives, these databases provide high-resolution, deeply curated data that facilitates specialized analysis. They are essential for researchers focusing on niche biological questions that require detailed, non-redundant, and expertly annotated information.

**48.** (C) DDBJ
The DNA Data Bank of Japan is the primary nucleotide sequence repository located in Japan and is a key member of the International Nucleotide Sequence Database Collaboration. DDBJ archives sequence data submitted by researchers, primarily from Asia, and synchronizes this information daily with GenBank and EMBL. This ensures that genomic data is globally integrated and freely accessible to everyone.

**49.** (A) P-3, Q-1, R-2, S-4
Bioinformatic data is stored in various standardized file formats. GenBank flat files are identified by a features table and an origin block. EMBL format uses two-letter line codes like ID and SQ. The PDB format contains detailed atomic coordinate records labeled ATOM and HETATM. FASTA files are characterized by a single description line starting with a greater-than symbol.

**50.** (B) I, II, III, IV and V
Sequence alignment relies on scoring matrices like PAM and BLOSUM. PAM matrices are based on observed mutations in related proteins, with higher numbers suited for divergent sequences. Conversely, BLOSUM matrices are derived from conserved blocks, with lower numbers used for distant relationships. BLOSUM62 is the common default for BLAST searches. A high PAM number generally corresponds to a low BLOSUM number.

**51.** (A) Substantially decreased computational time
The primary advantage of heuristic database search algorithms like BLAST over rigorous dynamic programming methods is the substantial reduction in computational time. While dynamic programming guarantees a mathematically optimal alignment, it is too slow for searching massive modern databases. Heuristic methods utilize seed-based strategies to rapidly identify significant similarities, making high-throughput genomic analysis feasible for researchers worldwide.

**52.** (B) tblastx
The tblastx program is a highly intensive BLAST variant that translates both a nucleotide query and a nucleotide database in all six reading frames. It then compares these protein translations against each other. This tool is particularly useful for identifying distant relationships between nucleotide sequences that might not be apparent at the DNA level due to codon degeneracy or evolutionary divergence.

**53.** (B) Guide tree
Progressive multiple sequence alignment tools utilize a preliminary computational structure known as a guide tree. This tree is constructed based on the pairwise distances between all sequences in the dataset. The algorithm then follows the branching order of the tree, aligning the most similar sequences first and progressively adding more divergent sequences to build the final multiple alignment result.

**54.** (C) III only
Phylogenetic construction methods have different assumptions and speeds. UPGMA assumes a constant rate of evolution, known as a molecular clock, whereas Neighbor-Joining does not. UPGMA is generally computationally faster than character-based methods like Maximum Parsimony, especially for large datasets. Therefore, the statement claiming Maximum Parsimony is faster than distance-based methods like UPGMA for large datasets is scientifically incorrect.

**55.** (B) Conserved protein sequences imply conserved genomic coding regions
Homology-based gene prediction is founded on the biological assumption that evolutionary conservation at the protein level reflects conservation in the underlying genomic coding regions. By identifying sequences in a novel genome that are highly similar to known genes in related species, bioinformaticians can accurately predict exon-intron structures. This method leverages established evolutionary relationships to facilitate genome annotation.

**56.** (D) Number of rotatable bonds
Lipinski's Rule of Five evaluates specific physicochemical properties to predict oral drug absorption. The parameters include molecular weight, the number of hydrogen bond donors, the number of hydrogen bond acceptors, and the calculated lipophilicity. While the number of rotatable bonds is an important factor in drug flexibility and bioavailability, it is not one of the criteria in the original Rule of Five.

**57.** (B) The 3D coordinate structure of the target macromolecule
Structure-based drug design is a targeted approach that fundamentally requires the three-dimensional coordinate structure of the macromolecular target, such as a protein or enzyme. This structural data, typically obtained through X-ray crystallography or NMR, allows researchers to visualize the active site. By understanding the spatial arrangement of atoms, they can design small molecules that precisely fit and inhibit the target.

**58.** (A) Binding free energy between the ligand and the receptor
In molecular docking, a scoring function is designed to estimate the binding free energy between a small molecule ligand and its target receptor. By calculating various physical interactions, such as hydrogen bonding and van der Waals forces, the function ranks different poses to identify the most stable orientation. This thermodynamic estimation is crucial for predicting the potency of drug candidates.

**59.** (C) 3D QSAR
3D QSAR models explicitly incorporate the three-dimensional spatial orientation, volumetric shape, and stereochemistry of molecules into their predictive framework. By analyzing interaction fields around aligned molecules, these models provide a detailed understanding of how spatial features influence biological activity. This approach is more sophisticated than 1D or 2D methods, offering greater insight for the optimization of potent drug candidates.

**60.** (A) Database growth outpaced rigorous dynamic programming speed.
The development of heuristic algorithms like FASTA and BLAST was necessitated by the fact that biological database growth rapidly outpaced the computational speed of rigorous dynamic programming methods. As the volume of genomic data increased exponentially, traditional algorithms became too slow for practical use. Heuristic approaches provided a necessary compromise, offering significant speed improvements while maintaining sufficient accuracy for most sequence searches.

**61.** (C) The interconnected architecture of biological neurons
Artificial Neural Networks are computational models designed to mimic the interconnected architecture and processing capabilities of biological neurons in the brain. They consist of layers of artificial nodes that process information and adjust their connection weights during training. In bioinformatics, ANNs are used for complex pattern recognition tasks, such as predicting protein secondary structures or classifying gene expression profiles.

**62.** (A) AlphaFold
AlphaFold is a groundbreaking deep learning platform that has revolutionized structural bioinformatics by predicting the three-dimensional structures of proteins with near-experimental accuracy. Developed by Google DeepMind, it utilizes sophisticated neural network architectures to interpret evolutionary and physical constraints from sequence data. This achievement addresses a long-standing challenge in biology, greatly accelerating research in drug discovery, enzyme design, and molecular biology.

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**63.** (B) Genomics, transcriptomics, and metabolomics
Omics data integration in veterinary medicine involves combining multiple distinct biological data layers to create a holistic model of animal health. By integrating genomics, transcriptomics, and metabolomics, researchers can better understand the complex interactions between genes, their expression, and the resulting metabolic products. This comprehensive approach is essential for identifying biomarkers and developing predictive models for complex diseases in livestock.

**64.** (A) I and III
The ExPASy Translate tool is a versatile resource for converting nucleotide sequences into their corresponding protein translations. It supports both the standard genetic code and various alternative codes used by organelles or specific organisms. Additionally, the tool can visually

utilizes branch lengths that are proportional to the magnitude of genetic divergence or time.

**29.** (A) Alignment → Distance matrix calculation → Star tree initialization → Node consolidation
The Neighbor-Joining method for phylogenetic tree construction follows a specific computational workflow. It begins with a multiple sequence alignment, followed by the calculation of a pairwise distance matrix. A star tree is then initialized, representing all taxa connected to a single central node. Finally, the algorithm iteratively consolidates nodes by identifying the closest neighbors to resolve the tree.

**30.** (A) I and II
Maximum Parsimony is a character-based phylogenetic method that operates on the principle that the simplest explanation is the most likely. It assumes the optimal evolutionary tree is the one requiring the minimum number of character changes to explain the observed data. However, it is not immune to long-branch attraction, an artifact that can lead to incorrect tree inferences.

**31.** (A) Statistical intrinsic signals such as codon bias, splice sites, and start/stop codons
Ab initio gene prediction algorithms identify potential coding regions by analyzing intrinsic genomic signals without comparing them to known sequences. These tools focus on statistical features such as codon usage bias, splice site motifs, and the presence of start or stop codons. By modeling these biological properties, the algorithms can predict gene locations in entirely novel genomic data.

**32.** (C) GeneMark
GeneMark is a specialized bioinformatics tool optimized for gene prediction in prokaryotic genomes. It utilizes inhomogeneous Markov models to differentiate between coding and non-coding DNA sequences. By accurately identifying species-specific patterns in nucleotide composition and codon usage, GeneMark provides high-resolution predictions of gene locations and operon structures in bacteria and archaea, facilitating rapid genome annotation.

**33.** (C) 500 Daltons
Lipinski's Rule of Five provides a set of criteria to evaluate the drug-likeness of chemical compounds. According to these guidelines, poor oral absorption and permeability are more likely if the molecular weight of a compound exceeds 500 Daltons. This threshold helps medicinal chemists prioritize small molecules with favorable pharmacokinetic profiles during the early stages of drug development.

**34.** (D) Excretion
In the field of pharmacokinetics, the acronym ADMET represents five key parameters essential for determining a drug's viability: Absorption, Distribution, Metabolism, Excretion, and Toxicity. The 'E' specifically stands for Excretion, which describes the biological processes by which a drug and its metabolites are eliminated from the body, typically through the kidneys in urine or through bile.

**35.** (A) Ligand-based drug design
Pharmacophore modeling is a central component of the ligand-based drug design paradigm. It involves identifying the common spatial arrangement of chemical features among a group of active molecules that are essential for their biological activity. This model is then used to screen libraries for new compounds that match the required pattern, even when the target structure is unknown.

**36.** (B) Flexible docking
Flexible docking is a sophisticated computational approach that evaluates multiple conformational states of both the small molecule ligand and the target protein's active site residues. By accounting for the inherent flexibility of biological molecules, this method provides a more accurate simulation of the binding process compared to rigid models, leading to better predictions of binding affinity.

**37.** (B) Lamarckian Genetic Algorithm
The AutoDock software package employs a Lamarckian Genetic Algorithm to explore the conformational space of a ligand during docking simulations. This hybrid approach combines the global search capabilities of genetic algorithms with a local search method. By allowing the best conformational poses to be inherited by the next generation, it efficiently identifies the most energetically favorable binding orientations.

**38.** (A) Correlate compound descriptors with biological activities.
The primary objective of developing a Quantitative Structure-Activity Relationship

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sequence alignment methods. It means that any gap insertion errors made during the initial pairwise alignments are fixed and cannot be removed or corrected in subsequent stages. This dependency on early decisions can lead to suboptimal alignments, especially when the initial sequences are highly divergent.

**74.** (A) I, II, III and V
Evolutionary trees can be rooted or unrooted. Unrooted trees show relationships without specifying an ancestor, while rooted trees provide a direction for evolutionary time. Outgroup selection is a common method for rooting. UPGMA naturally produces rooted trees due to its molecular clock assumption. However, unrooted trees actually contain fewer nodes than rooted ones for the same number of taxa.

**75.** (B) An explicit probabilistic model of sequence evolution
Maximum Likelihood phylogenetic construction differs fundamentally from Maximum Parsimony by utilizing an explicit probabilistic model of sequence evolution. While Parsimony seeks the tree with the fewest changes, Likelihood calculates the probability of the observed data given a specific evolutionary model and tree topology. This approach considers factors like varying substitution rates, providing a more robust statistical framework for tree inference.

**76.** (C) Inhomogeneous Markov chain models
The GeneMark algorithm is a powerful tool for gene prediction that utilizes inhomogeneous Markov chain models. These mathematical models capture the statistical differences in nucleotide patterns between coding and non-coding regions. By training on species-specific sequence data, GeneMark can accurately identify protein-coding genes in both prokaryotic and eukaryotic genomes, making it a cornerstone of modern genomic annotation pipelines.

**77.** (A) $\log P$
The molecular descriptor $\log P$ refers to the predicted partition coefficient of a chemical compound between an octanol phase and an aqueous phase. It serves as a measure of a molecule's lipophilicity or hydrophobicity. In drug design, $\log P$ is a critical parameter for estimating how easily a compound can penetrate lipid bilayers, such as cell membranes, influencing its overall bioavailability.

**78.** (A) A spatial arrangement of structural features essential for a molecule's biological activity
A pharmacophore is defined as the specific spatial arrangement of structural and chemical features within a molecule that are essential for its biological activity. These features, such as hydrogen bond donors or hydrophobic regions, interact with a target receptor to trigger a response. Identifying a pharmacophore allows researchers to search for new compounds that share the same essential binding characteristics.

**79.** (A) P-2, Q-1, R-4, S-3
Molecular docking uses various algorithms to find optimal binding poses. Monte Carlo methods randomly alter conformations and accept them based on probability. Genetic algorithms recombine features of successful poses. Simulated annealing uses a gradual cooling parameter to escape local energy minima. Incremental construction docks small fragments sequentially. These diverse strategies allow software to explore complex spaces and identify the most stable complexes.

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**80.** (A) Both A and R are true and R is the correct explanation of A.
3D-QSAR analysis requires the structural alignment or superposition of all training set molecules before interaction fields are calculated. This is because the descriptors in 3D-QSAR are spatially dependent and are evaluated on a 3D grid surrounding the molecules. Proper alignment ensures that the calculated steric and electrostatic fields are compared consistently across the entire series of chemical compounds.

**81.** (B) Excluding antigens that show homology to host proteins
In reverse vaccinology, a critical in silico screening criterion is the exclusion of antigens that share high homology with host proteins. This step is essential to prevent the development of autoimmune responses, where the vaccine-induced immune system mistakenly attacks the host's own tissues. By selecting only pathogen-specific sequences, researchers ensure the safety and specificity of the resulting vaccine candidates.

**82.** (A) NetMHC
NetMHC is a widely utilized immunoinformatics tool designed to predict the binding affinity of peptide sequences to various human leukocyte

high accuracy. Because prokaryotic genomes lack the complex intron-exon structures found in eukaryotes, Glimmer's algorithmic approach is particularly well-suited for the rapid annotation of these relatively compact genomes.

**101.** (A) Toxicity
In pharmacokinetics and drug discovery, the 'T' in the ADMET acronym stands for Toxicity. This parameter assesses the potential harmful effects a chemical compound may have on a biological system, including individual cells or whole organs. Evaluating toxicity early in the drug development pipeline is essential for ensuring patient safety and reducing the risk of failure during clinical trials.

**102.** (A) The drug exerts its effect by structurally binding to a specific pocket on the target
Structure-based drug design is predicated on the physical relationship that a drug candidate exerts its biological effect by binding to a specific pocket or active site on a target protein. By utilizing high-resolution three-dimensional structures, researchers can design molecules that complement the shape and chemical properties of the binding site, thereby inhibiting or modulating the target's function effectively.

**103.** (C) Neither the ligand nor the receptor can change conformation during the docking simulation
In molecular docking, a 'rigid body' approach refers to a simulation where both the ligand and the target receptor are kept in fixed, non-flexible conformations. This method significantly reduces computational complexity and time by only evaluating the translational and rotational degrees of freedom. While less realistic than flexible docking, it is often used for initial, rapid screenings of large compound libraries.

**104.** (A) I and II
QSAR model validation involves several statistical metrics to ensure reliability. R-squared ($R^2$) measures how well the model fits the training data, while Q-squared ($Q^2$) assesses the model's predictive power through cross-validation. A high $R^2$ does not automatically guarantee a high $Q^2$, as a model can be overfitted to the training set. Therefore, both parameters are necessary for proper evaluation.

**105.** (A) Conformational epitopes
B-cell epitopes that depend on the three-dimensional, folded structure of a protein to bring distant amino acids into spatial proximity are known as conformational epitopes. Unlike linear epitopes, which consist of a continuous sequence, conformational epitopes are formed by residues that are far apart in the primary sequence but become adjacent when the protein achieves its native tertiary fold.

**106.** (C) MHC / HLA complexes
The identification of immunogenic T-cell epitopes relies heavily on modeling the affinity with which a peptide fragment binds to the Major Histocompatibility Complex or human leukocyte antigen molecules. MHC complexes are responsible for presenting digested peptides on the cell surface, where they are recognized by T-cell receptors. Predicting this binding affinity is a cornerstone of modern vaccine design.

**107.** (B) Deep Learning
Deep learning is a machine learning approach that processes data through multiple layers of abstraction, mimicking the hierarchical structure of neural architectures in the brain. This methodology, which is a subset of artificial neural networks, is capable of learning complex features from raw data. It has been successfully applied in AlphaFold to achieve unprecedented accuracy in protein structure prediction.

**108.** (A) Genomic selection
Genomic selection is a technique used in animal breeding that integrates genome-wide marker data to predict the breeding value of an individual. Unlike traditional methods that rely solely on phenotypic observations, genomic selection allows for the early identification of superior animals based on their genetic makeup. This approach significantly accelerates genetic progress by reducing generation intervals and increasing the selection accuracy.

**109.** (A) P-4, Q-3, R-2, S-1
Different bioinformatics databases serve specific research needs. The NCBI Genome database focuses on complete chromosome assemblies and metadata. SCOP provides structural classifications based on evolutionary relationships. PIR is known for its curated protein superfamily classifications. TrEMBL archives unreviewed protein sequences that are

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dimensional atomic coordinates for standard amino acid residues and nucleic acids. These records provide the precise spatial location of each atom within the protein or DNA backbone and side chains. In contrast, HETATM records are used for non-standard atoms, such as ligands, water, or metal ions.

**92.** (C) Inverted indexes
The Sequence Retrieval System utilizes inverted indexes as its primary data structure to facilitate rapid querying across multiple disparate flat-file databases. An inverted index maps individual search terms to the specific records that contain them, allowing the system to locate information without scanning every file. This indexing strategy is essential for the efficient integration and retrieval of biological data from heterogeneous sources.

**93.** (A) Start codons and Stop codons
Open Reading Frame Finder tools identify potential protein-coding regions by scanning DNA sequences for segments bounded by specific functional codons. An ORF typically begins with a start codon, most commonly ATG, and continues until it reaches an in-frame stop codon. Identifying these segments is a fundamental step in gene prediction and the functional annotation of newly sequenced genomic DNA fragments.

**94.** (A) Local sequence alignment
The Smith-Waterman algorithm is a dynamic programming method that provides a mathematically guaranteed optimal result for local sequence alignments. Unlike global alignment methods, it identifies the best-matching sub-segments between two sequences, making it ideal for finding conserved domains or motifs within sequences of different lengths or overall low similarity. It remains a gold standard for sensitivity in sequence comparison.

**95.** (A) Less than or equal to 62% identity
The BLOSUM62 substitution matrix was derived from observations of protein sequence blocks sharing a specific identity threshold. It is based on alignments where sequences with more than 62% identity were clustered to reduce redundancy. This means the matrix represents observed substitutions in sequences that share 62% or less identity, making it highly effective for general-purpose protein database searches and alignments.

**96.** (B) The match is statistically highly significant and extremely unlikely to be due to chance
A BLAST E-value of 0.0 indicates that the observed sequence alignment is statistically highly significant. This value represents the number of hits one can expect to see by chance; therefore, a value of zero implies it is virtually impossible for such a match to occur randomly. This provides strong evidence that the sequences are homologous and share a common evolutionary origin.

**97.** (A) MUSCLE
MUSCLE is a widely used multiple sequence alignment program that employs an iterative approach to optimize a log-expectation objective function. It is designed for both speed and accuracy, often outperforming older progressive methods like ClustalW. By repeatedly refining the alignment and recalculating distances, MUSCLE can handle large datasets efficiently while producing high-quality alignments for complex protein and nucleotide families.

**98.** (A) Monophyletic
In molecular phylogeny and systematics, a monophyletic group, also known as a clade, is defined as a grouping that includes a single common ancestor and all of its descendants. This concept is fundamental to cladistics, as it represents a complete evolutionary lineage. Identifying monophyletic groups allows scientists to organize biological diversity based on shared ancestry rather than superficial similarities.

**99.** (A) Both A and R are true and R is the correct explanation of A.
Distance-based phylogenetic methods operate by compressing all sequence data into a single numerical value representing the genetic distance between pairs of taxa. While this approach allows for very rapid tree construction, it has the inherent limitation of losing information about specific, individual character changes. Consequently, these methods focus on overall similarity rather than the detailed evolutionary history of particular sites.

**100.** (D) Bacteria and Archaea
Glimmer is a highly efficient gene-finding software package primarily designed for predicting genes in the genomes of bacteria and archaea. It utilizes interpolated Markov models to identify potential protein-coding regions with

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antigen (HLA) molecules. By employing advanced machine learning algorithms, it estimates which peptides are most likely to be presented to T-cells. This capability is fundamental for identifying potential epitopes for vaccine design and understanding the cellular immune response to pathogens.

**83.** (C) Decision trees
The Random Forest machine learning algorithm classifies data by constructing an ensemble of multiple decision trees during the training process. Each tree in the forest makes a prediction, and the final classification is determined by a majority vote among all the individual trees. This approach enhances accuracy and prevents overfitting, making it highly effective for analyzing complex biological datasets with many variables.

**84.** (A) Single Nucleotide Polymorphism
In the context of genetics and bioinformatics, SNP stands for Single Nucleotide Polymorphism. It refers to a variation in a single nucleotide occurring at a specific position in the genome, where each variation is present to some appreciable degree within a population. SNPs are the most common type of genetic variation and are used as markers in animal breeding and disease studies.

**85.** (B) Poly-A tail
During the process of post-transcriptional modification in eukaryotic cells, a long chain of adenine nucleotides, known as a poly-A tail, is added to the 3-prime end of the mRNA molecule. This modification is crucial for protecting the mRNA from degradation, aiding in its transport from the nucleus to the cytoplasm, and enhancing its translation efficiency by the ribosome.

**86.** (B) DDBJ
The DNA Data Bank of Japan is the primary sequence database that curates and stores genetic data submitted by researchers in Japan. As a member of the International Nucleotide Sequence Database Collaboration, DDBJ shares all its records with GenBank and EMBL on a daily basis. This global cooperation ensures that all sequence information is available to the entire scientific community regardless of origin.

**87.** (B) Manual literature-based curation and biological annotation
Swiss-Prot distinguishes itself from the TrEMBL section of UniProt by its emphasis on manual, literature-based curation and extensive biological annotation. Each record is reviewed by expert biologists who integrate information from peer-reviewed research to ensure high data quality and reliability. In contrast, TrEMBL consists of unreviewed, computationally translated sequences that await the rigorous manual review process characteristic of Swiss-Prot.

**88.** (C) Class, Architecture, Topology, Homologous superfamily
The CATH classification system organizes protein structures into four hierarchical levels: Class, Architecture, Topology, and Homologous superfamily. Class is determined by the secondary structure content, while Architecture describes the overall shape. Topology refers to the specific fold and connectivity of structural elements, and Homologous superfamily identifies proteins with a clear common ancestor. This hierarchy provides a systematic framework for understanding structural relationships.

**89.** (A) GenBank → PROSITE → PDB
Biological databases are categorized based on the type of data they store. GenBank is a primary repository for raw nucleotide sequences. PROSITE is a secondary database that archives protein motifs and patterns derived from primary data. The Protein Data Bank is a structural database containing three-dimensional atomic coordinates of biological molecules. This sequence correctly identifies one database from each fundamental category.

**90.** (A) II, I, IV, III
Submitting a novel sequence to GenBank via the BankIt tool involves a specific procedural workflow. The process begins by providing essential metadata, including author and publication details. Next, the researcher uploads the sequence data in FASTA format. Biological annotations and feature keys are then added to describe the sequence's functional regions. Finally, the submission is completed, and a GenBank accession number is issued.

**91.** (A) ATOM
In a standard PDB coordinate file, the ATOM record type is specifically used to list the three-

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computationally translated from nucleotide records. Together, these resources provide a comprehensive view of biological data from genes to structures.

**110.** (D) Transcription
Transcription is the fundamental biological process that involves the synthesis of an RNA molecule from a DNA template. This process is carried out by the enzyme RNA polymerase, which reads the DNA sequence and assembles a complementary RNA strand. It represents the first stage of gene expression, where the genetic code is copied into a mobile form for protein synthesis.

**111.** (A) USA, Europe, Japan
The International Nucleotide Sequence Database Collaboration consists of three primary global members located in distinct regions. GenBank is maintained by the National Center for Biotechnology Information in the USA, EMBL-EBI is based in Europe, and the DNA Data Bank of Japan is located in Japan. These regions collaborate to maintain a comprehensive and synchronized repository of all DNA sequences.

**112.** (B) The time lag caused by the slow, manual curation process required for Swiss-Prot
The TrEMBL database was established to address the significant time lag caused by the manual curation process required for Swiss-Prot records. With the explosion of genomic data, it became impossible for human curators to keep pace with new sequence submissions. TrEMBL provides automated, computational translations of all coding sequences in the primary databases, ensuring that protein data is available immediately.

**113.** (C) SCOP
SCOP is a structural database that manually organizes proteins into a hierarchy of families, superfamilies, and folds. This classification is based on expert visual inspection and detailed analysis of evolutionary relationships between proteins. By grouping proteins with similar three-dimensional shapes and common ancestry, SCOP provides a reliable framework for understanding the structural diversity and evolution of the protein universe.

**114.** (A) I, II and IV
Genome browsers are essential for visualizing genomic data. The UCSC Genome Browser uses a track-based interface, and Ensembl provides extensive annotations for vertebrate genomes. The NCBI Genome Data Viewer also facilitates the visualization of sequence alignments. However, AnimalQTLdb focuses on quantitative trait loci rather than viral structural coordinates. Therefore, statements I, II, and IV are accurate descriptions of these tools.

**115.** (A) A federated network of distinctly maintained life science databases
When a researcher uses the Entrez interface, they are performing a search across a federated network of many distinctly maintained life science databases. Entrez serves as a global query system that integrates data from diverse sources, including nucleotide sequences, protein structures, and scientific literature. This integrated approach allows for seamless cross-referencing and comprehensive retrieval of biological information from a single platform.

**116.** (C) An ambiguous or unknown nucleotide
In the FASTA file format, the character 'N' is used within a nucleotide sequence to represent an ambiguous or unknown base. This typically occurs when sequencing technology cannot definitively identify a specific nucleotide at a particular position. Bioinformatics tools are designed to handle these 'N' characters appropriately during sequence analysis, alignment, and genome assembly processes to ensure data integrity.

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**117.** (A) Preparing complex sequence submissions for GenBank
Sequin was a widely utilized standalone software tool designed specifically for preparing complex sequence submissions for the GenBank database. It allowed researchers to add detailed biological annotations, feature keys, and metadata to their sequences before submission. While it has largely been replaced by web-based tools like BankIt, it was essential for managing large-scale genomic data and ensuring high-quality database entries.

**118.** (A) Accumulating gap penalties linearly
In a dynamic programming matrix for global sequence alignment, such as the Needleman-Wunsch algorithm, the first row and column are initialized by accumulating gap penalties linearly. This process reflects the increasing cost

of aligning a sequence against a series of gaps. This initialization is critical for ensuring the algorithm correctly calculates the optimal global alignment score over the entire sequence length.

**119.** (B) 1 accepted point mutation per 100 amino acids
The PAM1 substitution matrix represents a specific unit of evolutionary divergence. It corresponds to an evolutionary distance where one accepted point mutation has occurred per 100 amino acid residues. Derived from the analysis of closely related proteins, PAM1 serves as the basis for calculating other PAM matrices, like PAM250, by extrapolating the probability of mutations over longer periods.

**120.** (A) $G = o + e \times L$
The affine gap penalty model is mathematically defined by the formula $G = o + e \times L$, where $G$ is the total gap penalty. In this equation, '$o$' represents the penalty for opening a new gap, '$e$' is the penalty for extending an existing gap, and '$L$' is the length of the gap. This model provides a more biologically realistic scoring system.

**121.** (B) Identifying short, exact word matches (seeds) between query and database
The BLAST heuristic algorithm accelerates database searches by starting with a specific computational step. It identifies short, exact word matches, known as seeds, between the query sequence and the database. These initial matches must score above a certain threshold. Once a seed is found, the algorithm attempts to extend it to find a high-scoring segment pair that represents a significant alignment.

**122.** (A) It improves accuracy for globally divergent sequences.
T-Coffee offers a significant advantage in multiple sequence alignment by improving accuracy for globally divergent sequences. By building an initial library that combines both global and local pairwise alignment information, it captures a broader range of evolutionary signals. This approach helps the algorithm avoid the common errors associated with traditional progressive methods, leading to more reliable alignments for distantly related proteins.

**123.** (C) Paralogs
Paralogs are homologous genes that result from a gene duplication event within a single evolutionary lineage. Following duplication, one copy of the gene may evolve a new biological function while the other retains the original role. Identifying paralogous relationships is important for understanding how new functions arise and for correctly interpreting gene families and their evolutionary history within a specific species.

**124.** (A) II, III and IV
The Neighbor-Joining method is a popular distance-based phylogenetic algorithm. It constructs unrooted trees and works by attempting to minimize the total sum of branch lengths at each stage of node consolidation. NJ is statistically robust and efficient for large datasets. However, it is not a character-based method and does not assume a strict molecular clock, unlike the UPGMA method.

**125.** (B) Long-branch attraction
Long-branch attraction is a statistical artifact that frequently causes the Maximum Parsimony method to infer incorrect phylogenetic trees. It occurs when certain branches in the tree have evolved exceptionally rapidly, leading to coincidental similarities between unrelated taxa. Parsimony mistakenly interprets these random similarities as shared derived characters, resulting in the grouping of long branches together regardless of true relationship.

**126.** (A) Exon-intron boundaries (splice sites)
In eukaryotic gene prediction, ab initio algorithms must accurately model exon-intron boundaries, also known as splice sites, which are completely absent in prokaryotes. Eukaryotic genes are interrupted by non-coding regions, requiring sophisticated methods to identify donor and acceptor sites for proper transcript assembly. This structural complexity significantly increases the difficulty of gene prediction compared to the continuous coding regions in bacteria.

**127.** (A) 5
According to Lipinski's Rule of Five, an ideal oral drug candidate should generally possess no more than five hydrogen bond donors. A hydrogen bond donor is defined as the sum of all nitrogen-hydrogen and oxygen-hydrogen bonds in the molecule. Staying within this threshold is important for maintaining favorable permeability and absorption characteristics, which are essential for a successful pharmaceutical product.

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**128.** (A) Low molecular weight and low binding affinity
Fragment-based drug design initiates by identifying very small chemical compounds, known as fragments, characterized by low molecular weight and low binding affinity. Although these fragments bind weakly to the target, they form high-quality interactions within the active site. These small building blocks are then iteratively optimized or linked together to create larger molecules with significantly higher potency and selectivity.

**129.** (A) Both A and R are true and R is the correct explanation of A.
Flexible ligand docking is computationally more intensive than rigid body docking because it must evaluate a vast number of potential torsional rotations and conformational states. While rigid docking only considers movement as a single unit, flexible docking accounts for the internal movements of the ligand's bonds. This increased complexity is necessary to accurately simulate how a drug molecule adapts its shape.

**130.** (A) I and II
Molecular docking utilizes different types of scoring functions to evaluate binding. Empirical scoring functions use terms based on binding energy components like hydrogen bonds and hydrophobics. Knowledge-based functions derive potentials from statistical analysis of experimentally determined crystal structures. However, force-field methods do not ignore electrostatic interactions; they typically include them as a major component of the potential energy calculation.

**131.** (A) Convert chemical structures into mathematical variables suitable for statistical modeling
The primary purpose of calculating molecular descriptors in QSAR modeling is to convert complex chemical structures into discrete mathematical variables. These numerical representations describe various physical, chemical, and topological properties of the molecules. By transforming structural data into a format suitable for statistical analysis, researchers can build predictive models that correlate chemical features with observed biological activities.

**132.** (B) Experimentally validated MHC-binding peptides
In silico T-cell epitope prediction frequently relies on position-specific scoring matrices that are derived from large datasets of experimentally validated MHC-binding peptides. These matrices capture the frequency of each amino acid at specific positions within the peptide, identifying the residue preferences for binding to different MHC alleles. This statistical information is then used to scan novel sequences for potential immunogenic regions.

**133.** (C) Random Forest
Random Forest is an ensemble machine learning algorithm that operates by building multiple decision trees during the training phase. For classification tasks, the algorithm outputs the class that represents the mode of the individual trees' predictions. By combining the results of many different trees, Random Forest improves overall accuracy and robustness, effectively handling noise and high-dimensional data in various applications.

**134.** (A) Classifying disease states based on complex gene expression microarray patterns
Artificial Neural Networks are applied in transcriptomics for tasks like classifying disease states based on complex gene expression patterns from microarrays or RNA-seq data. ANNs can identify subtle, non-linear relationships within these large datasets that traditional statistical methods might miss. This allows for the development of highly accurate diagnostic models and the identification of gene signatures associated with specific conditions.

**135.** (C) Real-time monitoring and management of animal health, welfare, and production
Precision livestock farming leverages bioinformatics, sensors, and data integration to achieve real-time monitoring and management of animal health, welfare, and production. By continuously collecting data on animal behavior, physiology, and environmental conditions, farmers can make informed decisions to optimize production efficiency and ensure the well-being of their livestock. This technology-driven approach is essential for modern agriculture.

**136.** (C) Protein
The Central Dogma of molecular biology emphasizes that once genetic information has

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been translated into a protein, it cannot flow back to nucleic acids like DNA or RNA. This principle highlights the unidirectional nature of the translation process, where the sequence of amino acids is determined by the nucleotide sequence, but the protein cannot alter the genetic code itself.

**137.** (D) GenPept
GenPept is a specialized protein sequence database that consists of amino acid sequences derived from the automated translation of all coding sequences found in the GenBank, EMBL, and DDBJ nucleotide repositories. It serves as a comprehensive resource for researchers looking for protein information associated with specific DNA records, providing a direct link between genomic sequences and their potential protein products.

**138.** (D) Sequence Read Archive (SRA)
The Sequence Read Archive is the primary repository at the National Center for Biotechnology Information for the submission of raw, unaligned next-generation sequencing reads. It stores massive datasets from various high-throughput sequencing platforms, ensuring that the original data is preserved for future re-analysis and validation. This archive is essential for transparency and data sharing in the genomic research community.

**139.** (A) P-2, Q-1, R-4, S-3
Bioinformatics tools are categorized by their primary research focus. Swiss-Prot provides curated protein sequences with detailed annotations. AlphaFold is renowned for protein structure prediction using deep learning. Clustal Omega is a standard tool for performing multiple sequence alignments. GeneMark is an ab initio gene prediction program used for identifying coding regions. These resources are fundamental for different stages of biological data analysis.

**140.** (A) I, II and III
The CATH classification system uses a hierarchical approach to organize protein structures. Class is determined by secondary structure content, Architecture describes the macroscopic arrangement of these structures, and Topology considers their specific connectivity and fold. While many steps are automated, CATH does not rely exclusively on manual curation; it utilizes algorithms like DALI and SSAP to aid in the structural classification process.

**141.** (B) Polygenic traits like milk yield and disease resistance.
AnimalQTLdb is an invaluable resource for mapping polygenic traits in livestock, such as milk yield, growth rates, and disease resistance. These traits are controlled by multiple genes and are influenced by the environment. By archiving associations between genetic markers and these quantitative phenotypic traits, the database supports the development of genomic selection models and marker-assisted breeding strategies in veterinary science.

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**142.** (C) It narrows search space to improve result relevance.
The Entrez database interface allows researchers to utilize 'Limits' or 'Filters' to narrow the search space, which significantly improves the relevance of the retrieved results. By specifying parameters like organism, date of publication, or molecule type, users can filter out unwanted records and focus on the most pertinent information. This computational indexing strategy makes searching massive biological databases fast and efficient.

**143.** (B) PDB
The PDB file format is the strictly standardized representation for three-dimensional atomic coordinates of biological macromolecules. It contains detailed information about the spatial position of every atom in a protein or nucleic acid structure. Because of its precision, the PDB format is heavily utilized in molecular docking, structural analysis, and simulations to study the physical interactions between molecules.

**144.** (A) III, I, IV, II
An effective homology-based BLAST search follows a specific procedural sequence. First, the researcher inputs the query sequence in FASTA format. Next, the appropriate BLAST variant, such as blastp for proteins, is selected. The target database, like the non-redundant 'nr' database, is then chosen. Finally, the search is executed, and the resulting E-values and bit scores are interpreted.

**145.** (A) Both A and R are true and R is the correct explanation of A.
The ExPASy Translate tool generates six distinct translation frames for any given DNA

sequence. This is because double-stranded DNA possesses three forward reading frames on the sense strand and three reverse reading frames on the complementary antisense strand. Since the correct frame is not always known beforehand, evaluating all six possibilities is necessary to identify the actual protein-coding region.

**146.** (B) Normalized to be independent of the specific scoring matrix used
In sequence alignment, the bit score is distinguished from the raw score by being normalized to be independent of the specific scoring matrix and database size. This normalization allows for the direct comparison of results from different searches. A higher bit score indicates a better alignment, providing a more universal and reliable measure of the quality of the match between sequences.

**147.** (C) Highly divergent (distant evolutionary relationships)
The BLOSUM45 substitution matrix is mathematically tailored for aligning sequences that are highly divergent and share distant evolutionary relationships. It is derived from sequence blocks that share 45% or less identity. By focusing on substitutions observed in more distantly related proteins, BLOSUM45 provides greater sensitivity when searching for ancient homologs compared to matrices derived from more closely related sequences.

**148.** (B) An introduced space representing an evolutionary insertion or deletion event
In the context of sequence alignments, a gap is defined as an introduced space representing an evolutionary insertion or deletion event, collectively known as an indel. Gaps are used to align sequences of different lengths to account for residues that have been lost or gained over time. Proper gap placement is essential for accurately identifying homologous regions and calculating scores.

**149.** (B) $K$-tuples or words
The FASTA algorithm accelerates sequence alignments by initially locating identical matches of a specific length between two sequences. These exact matches are called $k$-tuples or words. By identifying these regions of absolute identity first, the algorithm can quickly focus on areas of potential similarity before performing more rigorous and time-consuming dynamic programming to refine the final alignment result.

**150.** (A) I, II, IV and V
Multiple Sequence Alignment methods have different strengths. Iterative methods like MUSCLE refine alignments by realigning subgroups, and Clustal Omega handles massive datasets using Hidden Markov Models. MSA is also a crucial prerequisite for phylogenetic construction. However, progressive alignments are not mathematically guaranteed to find the absolute optimal result due to their susceptibility to early errors during the guide tree building process.

**151.** (C) Xenologs
Xenologs are a specific type of homologous genes that are acquired by a genome through horizontal gene transfer between different, often distantly related, species. Unlike orthologs and paralogs, which arise through vertical inheritance and speciation or duplication, xenologs represent the movement of genetic material across taxonomic boundaries. Identifying xenologs is essential for understanding microbial evolution and the spread of functional traits.

**152.** (B) UPGMA and Neighbor-Joining
Distance-based phylogenetic construction algorithms include two widely utilized methods: UPGMA and Neighbor-Joining. UPGMA is a simple clustering method that assumes a constant rate of evolution, while Neighbor-Joining is a more robust approach that does not make this assumption. Both methods transform a pairwise distance matrix into a branching tree, providing a rapid way to visualize evolutionary relationships among different biological taxa.

**153.** (B) Maximum Likelihood
Maximum Likelihood is a phylogenetic construction method that infers an evolutionary tree by maximizing the probability of observing the given sequence alignment under a specific model of evolution. By evaluating different topologies and branch lengths, the algorithm identifies the tree that makes the observed data most likely. This statistically grounded approach is highly sensitive and widely used in molecular phylogenetics research.

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**154.** (B) Bootstrapping
Bootstrapping is the computational metric most commonly utilized to assess the statistical confidence of individual branches within a phylogenetic tree. This technique involves creating multiple pseudo-replicate datasets by randomly resampling the original sequence alignment. The frequency with which a specific branch appears in the trees generated from these replicates is reported as a percentage, indicating its reliability and statistical support.

**155.** (B) Ab initio gene prediction
Ab initio gene prediction refers to the process of identifying potential genes purely based on intrinsic genomic signals, such as open reading frames and CpG islands. This method does not rely on comparing the sequence to known transcripts or homologs in other species. Instead, it uses statistical models to recognize patterns characteristic of coding regions, making it essential for annotating novel genomes.

**156.** (D) General Hidden Markov Models (GHMM)
The gene prediction tool AUGUSTUS relies on the General Hidden Markov Models framework to improve the accuracy of finding genes in eukaryotic genomes. This sophisticated mathematical architecture allows the software to model complex structural features like exons, introns, and splice sites simultaneously. By incorporating various genomic signals into a probabilistic model, AUGUSTUS provides highly reliable predictions for a wide range of biological species.

**157.** (A) Metabolism
The ADMET property that dictates how a pharmaceutical compound is enzymatically altered within the liver is Metabolism. This process involves the chemical transformation of the drug by enzymes, which can either activate a pro-drug or lead to the detoxification and elimination of a substance. Understanding metabolic pathways is crucial for predicting a drug's halF-life, interactions, and potential side effects in patients.

**158.** (B) Molecular fingerprint
A molecular fingerprint is a type of descriptor that encodes the presence or absence of specific structural fragments within a chemical molecule as a binary string of zeros and ones. This representation allows for rapid comparison and searching of large chemical databases based on structural similarity. Molecular fingerprints are essential tools in chemoinformatics for virtual screening and identifying potential drug candidates.

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**159.** (B) Hydrogen bond donor
In pharmacophore modeling, a hydrogen bond donor is considered a standard pharmacophoric feature. These features represent specific chemical groups that are capable of forming essential interactions with a target receptor. Other common features include hydrogen bond acceptors, hydrophobic regions, and aromatic rings. Identifying these features and their spatial arrangement is key to understanding how molecules bind and exert their effects.

**160.** (A) P-2, Q-3, R-4, S-1
QSAR models are classified by their dimensionality and descriptor types. 1D QSAR uses global properties like molecular weight. 2D QSAR employs topological indices based on atom connectivity. 3D QSAR analyzes spatial interaction fields around aligned molecules. 4D QSAR goes further by incorporating conformational ensembles and multiple structural states. This hierarchy reflects the increasing complexity and detail of the molecular information utilized.

**161.** (C) Incremental Construction
The Incremental Construction algorithm is a molecular docking search strategy that sequentially places small molecular fragments into the binding site and then dynamically links them to form the complete ligand. This approach efficiently explores the conformational space by building the molecule piece by piece within the constraints of the protein's active site, allowing for the identification of favorable binding poses.

**162.** (B) Rino Rappuoli
Rino Rappuoli pioneered the approach known as reverse vaccinology by successfully applying it to the pathogen Neisseria meningitidis serogroup B. By using the complete genomic sequence to identify potential vaccine candidates in silico, he bypassed traditional culture-based methods. This breakthrough transformed vaccine research, enabling the rapid discovery of protective antigens for many pathogens that were previously difficult to target.

**163.** (D) Hydrophilicity
Hydrophilicity is the physicochemical property most strongly correlated with antigenic regions in B-cell epitope prediction. Propensity scales are used to identify sequences that are likely to be surface-exposed and accessible to antibodies. Because B-cell epitopes must interact with large protein molecules in an aqueous environment, highly hydrophilic regions on the exterior of a folded protein are prime candidates for immunogenicity.

**164.** (B) Kernel trick
Support Vector Machines classify complex biological data by mapping inputs into high-dimensional feature spaces using a mathematical technique known as the kernel trick. This approach allows the SVM to find an optimal linear separating hyperplane in a space where the data might not be linearly separable in its original form. It is highly effective for tasks like protein classification and gene prediction.

**165.** (A) Evoformer (Transformer-based architecture)
The AlphaFold architecture drastically improves the accuracy of 3D protein structure prediction by utilizing a specific deep learning module called the Evoformer. This transformer-based architecture is designed to simultaneously process information from multiple sequence alignments and pair-wise residue relationships. By effectively capturing co-evolutionary and spatial constraints, the Evoformer enables the model to reason about complex structural folding.

**166.** (B) It significantly decreases the generation interval
In precision livestock farming, the integration of genomic selection models significantly decreases the generation interval. Traditional breeding requires waiting for animals to reach maturity and display phenotypic traits before selection. Genomic selection allows breeders to predict the genetic merit of young animals shortly after birth using DNA markers. This acceleration leads to faster genetic improvement and more efficient breeding programs.

**167.** (A) RNA virus replication via RNA-dependent RNA polymerase
A major exception to the classical Central Dogma is the selF-replication of RNA viruses, which does not involve a DNA intermediate. These viruses utilize a specialized enzyme called RNA-dependent RNA polymerase to synthesize new RNA strands directly from an RNA template. This mechanism allows them to bypass the host's normal transcription machinery, demonstrating that genetic information can flow between RNA molecules.

**168.** (B) Relational databases
Relational databases are a specific biological database structure that natively supports rapid, complex queries and minimizes data redundancy through the use of linked relational tables. By organizing data into distinct entities and defining relationships between them, these systems provide a powerful and efficient way to manage large-scale biological information, such as genomic sequences and phenotypic data.

**169.** (A) It aggregate and cross-references data from multiple distinct primary and secondary databases
The defining characteristic of a composite biological database is that it aggregates and cross-references data from multiple distinct primary and secondary databases. Instead of being a source for new experimental results, it provides a comprehensive, integrated view of existing biological information. This allows researchers to access a wide range of data, from sequences to structures and functions, through a single interface.

**170.** (C) TrEMBL records are uncurated computational translations.
Within the UniProt Knowledgebase, the primary feature that identifies a TrEMBL record compared to a Swiss-Prot record is that TrEMBL entries are uncurated computational translations. They are automatically generated from all available coding sequences in nucleotide databases and have not yet undergone the rigorous manual review and functional annotation process by expert curators that is characteristic of Swiss-Prot entries.

**171.** (A) X-ray crystallography, NMR spectroscopy, and Cryo-Electron Microscopy
The Protein Data Bank accepts three-dimensional structural data generated by three primary experimental techniques: X-ray crystallography, NMR spectroscopy, and Cryo-Electron Microscopy. These methods provide high-resolution information about the atomic

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data with extensive annotations and metadata, ensuring the submission meets GenBank's rigorous standards. They provide the necessary functionality for managing complex biological features and genomic assemblies.

**226.** (B) Superimposed layers of biological annotation aligned to a reference genome
In the UCSC Genome Browser, distinct 'tracks' correspond to superimposed layers of biological annotation that are precisely aligned to a reference genome sequence. These tracks can represent a wide variety of data, including gene models, regulatory elements, sequence conservation, and expression levels. This visual integration allows researchers to study the complex functional landscape of the genome within a single intuitive interface.

**227.** (A) Indexing the flat files while leaving the raw data in its native format.
In the Sequence Retrieval System, the ability to search efficiently across disparate databases is governed by indexing the flat files while leaving the raw data in its native format. By creating centralized indices for various data fields, SRS allows for rapid querying without the need for time-consuming data conversion or warehousing. This approach enables the integration of heterogeneous biological data resources.

**228.** (A) A stop codon
In the standard FASTA format, an asterisk character at the end of an amino acid sequence explicitly denotes a stop codon. This symbol indicates the termination of the protein-coding sequence. It is often used in translated sequences to show where the open reading frame ended. Bioinformatics software recognizes this character to correctly identify the completion of a polypeptide chain during sequence analysis.

**229.** (A) ClustalW
The BioEdit software package integrates a local implementation of the powerful ClustalW algorithm for performing multiple sequence alignments. BioEdit provides a graphical user interface that simplifies the execution of this complex task, allowing researchers to align multiple protein or nucleotide sequences easily. This integration makes BioEdit a versatile tool for sequence manipulation, alignment editing, and subsequent evolutionary analysis within Windows.

**230.** (A) I, II and III
The BLAST algorithm includes several variants for different search tasks. Blastn compares a nucleotide query against a nucleotide database. Blastp is used to search a protein database with a protein query. Tblastn translates a nucleotide database in all six reading frames to compare it against a protein query. All three statements correctly identify the function and data types for these specific BLAST programs.

**231.** (A) The E-value threshold
In heuristic database searches like BLAST, the E-value threshold is the parameter that dictates the minimum statistical significance required for a sequence match to be reported. By setting a specific threshold, researchers can control the number of hits returned, focusing on those most likely to be biologically relevant. Lowering the threshold increases search stringency, while raising it allows for more distant matches.

**232.** (A) A single contiguous multi-residue insertion is more probable than multiple independent single-residue insertions.
The affine gap penalty model addresses the biological observation that a single contiguous multi-residue insertion or deletion event is much more probable than multiple independent single-residue mutations. By setting the cost of opening a gap higher than the cost of extending it, the algorithm produces alignments that favor fewer, longer gaps, which more accurately reflects the mechanisms of biological evolution.

**233.** (A) Replaced with zero
The Smith-Waterman algorithm identifies optimal local alignments by utilizing a dynamic programming matrix where all negative cell scores are replaced with zero. This prevents poorly matching regions from accumulating negative values and lowering the overall score of a potential local match. This zero-thresholding is the key feature that allows the algorithm to find significant local similarities between sequences of any length.

**234.** (A) Progressive method (e.g., ClustalW)
Progressive multiple sequence alignment methods, such as ClustalW, are characterized by a strategy that strictly prevents the iterative realignment of sequences once they have been grouped according to the guide tree. Once an alignment is formed at a node, it remains fixed.

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order. Finally, it performs the progressive multiple alignment by following the branching pattern of the guide tree from the leaves.

**181.** (B) A taxon diverged prior to the ingroup's common ancestor.
In molecular phylogeny, an outgroup is defined as a taxon that diverged prior to the common ancestor of the group under study, known as the ingroup. The outgroup serves as a reference point, allowing researchers to determine the direction of evolutionary change and to root the phylogenetic tree. Selecting an appropriate outgroup is essential for correctly identifying ancestral versus derived character states.

**182.** (A) Maximum Parsimony
Maximum Parsimony is a character-based phylogenetic construction methodology that evaluates each nucleotide or amino acid position individually. Unlike distance-based methods that aggregate information into a single value, parsimony tracks the changes at each site across the tree. It identifies the tree topology that requires the minimum total number of character state changes, providing a detailed view of the evolutionary history.

**183.** (B) It explicitly assumes a strict molecular clock across all lineages
The principal limitation of the UPGMA phylogenetic tree construction method is its explicit assumption of a strict molecular clock across all lineages. This means it assumes that the rate of evolution is constant for all branches. If this assumption is violated, which is common in many biological systems, UPGMA can produce incorrect tree topologies. Consequently, more robust methods like Neighbor-Joining are preferred.

**184.** (C) Spidey
Spidey is a highly recognized bioinformatics tool used for performing spliced alignments, where cDNA transcripts are aligned to genomic DNA to identify exon-intron structures. By specifically accounting for the gaps created by introns, Spidey allows researchers to accurately map transcripts to their genomic origins. This homology-based approach is fundamental for identifying gene boundaries and understanding the structural organization of eukaryotic genomes.

**185.** (B) 5
According to Lipinski's Rule of Five, an ideal oral drug candidate should possess a calculated $\log P$ value, also known as $c\log P$, that does not exceed 5. This value represents the molecule's lipophilicity. Compounds with a $c\log P$ higher than 5 are often too hydrophobic, which can lead to poor solubility and low absorption in the gastrointestinal tract, hindering their pharmaceutical effectiveness.

**186.** (A) De novo structure-based drug design
De novo structure-based drug design is a methodology that attempts to construct a novel drug molecule from scratch within the constraints of a target's active site. Instead of screening existing libraries, researchers build the molecule atom-by-atom or fragment-by-fragment to perfectly complement the binding pocket's shape and chemical properties. This approach allows for the creation of highly specific and potent inhibitors.

**187.** (B) Computational speed and multi-threading capabilities
AutoDock Vina is renowned for significantly improving the computational speed and multi-threading capabilities compared to older versions of AutoDock. By utilizing a more efficient scoring function and search algorithm, Vina can perform molecular docking simulations much faster while maintaining high accuracy. This advancement makes it practical to conduct large-scale virtual screening of compound libraries, accelerating the drug discovery process.

**188.** (A) 2D
In QSAR modeling, topological indices are derived directly from the connection table of atoms and bonds, representing 2D descriptors. Examples include the Wiener index and molecular connectivity indices. These descriptors capture information about the branching and connectivity of the molecule without requiring information about its three-dimensional shape. 2D descriptors are computationally efficient and widely used for predicting physicochemical and biological properties.

**189.** (A) The rapid, cost-effective triaging of thousands of potential epitopes
A key advantage of in silico immunoinformatics over traditional laboratory screening is the ability to perform rapid and cost-effective triaging of

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thousands of potential epitopes. Computational methods can quickly predict MHC binding affinities and identify candidate antigens, allowing researchers to focus their experimental efforts on the most promising targets. This greatly accelerates vaccine development and reduces the overall cost and time.

**190.** (A) CD8+ Cytotoxic T-cells
Major Histocompatibility Complex Class I molecules are responsible for presenting endogenous peptide fragments to CD8+ Cytotoxic T-cells. These peptides are typically derived from proteins synthesized within the cell, including viral proteins during an infection. The recognition of these MHC-peptide complexes by Cytotoxic T-cells is a fundamental component of the adaptive immune response, leading to the destruction of infected cells.

**191.** (B) Backpropagation
Artificial Neural Networks employ the backpropagation algorithm to systematically minimize classification error during the training phase. This method works by calculating the gradient of the error function with respect to the network's weights and then adjusting those weights in the opposite direction. By iteratively repeating this process, the network 'learns' to identify patterns in the data, improving its predictive accuracy.

**192.** (A) K-Nearest Neighbors
The K-Nearest Neighbors algorithm is a simple yet effective machine learning method that classifies a data point based on the majority vote of the '$k$' most similar instances in the feature space. By calculating the distance between the query point and all other points in the training set, KNN identifies the nearest neighbors and assigns the most common label among them.

**193.** (A) Metabolomics
Metabolomics is the omics tier that directly studies the complete set of small-molecule intermediates and products of metabolism, known as the metabolome. In veterinary predictive disease modeling, metabolomics provides a functional readout of an animal's physiological state. By analyzing changes in metabolite levels, researchers can identify biomarkers for disease, monitor health, and understand the impact of nutrition on production.

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**194.** (C) Anticodon loop
The anticodon loop is the specific region of a tRNA molecule that physically base-pairs with a complementary codon on the mRNA strand during the process of translation. This interaction ensures that the correct amino acid, which is attached to the tRNA's acceptor stem, is added to the growing polypeptide chain. The anticodon-codon pairing is fundamental to the fidelity of genetic code translation.

**195.** (A) A boutique or specialized database (e.g., OMIM)
A biological database that meticulously curates sequence variations and correlates them strictly with inherited human diseases, such as OMIM, is best classified as a boutique or specialized database. These resources focus on high-quality, expert-level annotation within a narrow domain, providing comprehensive clinical and genetic information. They are essential tools for researchers and clinicians seeking to understand the genetic basis of health conditions.

**196.** (A) Nucleotide sequence submissions to any member are automatically mirrored to the others daily.
The fundamental agreement binding the INSDC consortium requires that all nucleotide sequence submissions made to any one member database are automatically mirrored to the others on a daily basis. This ensures that GenBank, EMBL, and DDBJ maintain identical sets of publicly available sequence data. This global synchronization is crucial for providing the scientific community with comprehensive and up-to-date access to genomic data.

**197.** (B) TrEMBL
The TrEMBL division of UniProt consists of millions of automatically annotated protein records that are waiting for manual review by expert curators. These records are derived from the computational translation of coding sequences in nucleotide databases. Once a TrEMBL entry is manually reviewed and annotated with information from the scientific literature, it is promoted to the Swiss-Prot section of the database.

**198.** (C) Topology
The CATH classification system employs the DALI algorithm extensively at the Topology level to detect structural similarities between protein

domains. Topology, also known as the 'fold' level, considers the specific spatial arrangement and connectivity of secondary structure elements. Using automated algorithms at this stage allows for the efficient grouping of proteins that share similar structural architectures, even if their sequences have diverged.

**199.** (A) RefSeq genomes
The NCBI Genome database organizes high-throughput sequencing data into RefSeq genomes, which represent a refined, well-annotated reference standard. RefSeq provides a stable and non-redundant set of records for genes, transcripts, and proteins. These reference sequences are essential for consistent genome analysis, serving as the foundation for comparative genomics, variant calling, and functional studies across a wide variety of biological organisms.

**200.** (A) Both A and R are true and R is the correct explanation of A.
The Entrez system utilizes inverted indices to accelerate complex Boolean queries across its distinct biological databases. An inverted index works by mapping individual terms to the specific records that contain them, bypassing the need to read every document during a query. This indexing strategy is the key to Entrez's ability to provide rapid and integrated search results from millions of biological records.

**201.** (B) SOURCE
In a GenBank flat file, the SOURCE field provides metadata regarding the biological organism from which the sequence was derived. This includes the common name and the full taxonomic classification of the species. This information is critical for researchers to identify the origin of the sequence data and to ensure it is correctly associated with the appropriate evolutionary lineage during comparative analysis.

**202.** (A) I only
The ExPASy Translate tool correctly addresses the biological fact that mitochondrial genomes often utilize variants of the standard genetic code. However, eukaryotic translation still relies on standard start codons, and while splice sites determine the mRNA sequence, they do not 'dynamically alter' the frame during translation itself. Furthermore, bacterial genomes do contain stop codons. Thus, only the first statement is accurate.

**203.** (C) Affine gap penalties
In sequence alignment, the affine gap penalty is the parameter that prevents the accumulation of biologically unrealistic, continuous gaps. By applying a higher cost to opening a gap than to extending one, the algorithm favors single long gaps over many short ones. This approach more accurately reflects the evolutionary process of insertions and deletions, leading to alignments that are more biologically meaningful.

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**204.** (B) Closely related
In the derivation of BLOSUM matrices, a higher matrix number, such as BLOSUM80, indicates that the matrix is tailored for comparing sequences that are closely related. These matrices are derived from sequence blocks sharing a high percentage of identity. Conversely, lower-numbered matrices like BLOSUM45 are better suited for identifying distant homologs, as they represent observed substitutions in more divergent sequences.

**205.** (C) $K$-tuples
The FASTA heuristic database search tool accelerates sequence alignments by initially locating identical regions called $k$-tuples. These are short, exact matches of a specified length between the query and the database sequences. By identifying these seeds first, the algorithm can rapidly filter the database for potentially significant matches before applying more rigorous dynamic programming methods to determine the final optimal alignment.

**206.** (A) Re-evaluating and correcting errors made during initial pairwise alignments.
Iterative multiple sequence alignment methods, such as MUSCLE, improve upon traditional progressive methods by re-evaluating and correcting errors made during the initial stages. Unlike progressive methods that fix alignments once they are formed, iterative approaches repeatedly refine the alignment to optimize an objective function. This leads to higher-quality results, particularly for complex datasets where early mistakes in a guide tree can propagate.

**207.** (A) Clade
In phylogenetic terminology, a monophyletic group is completely synonymous with the term

arrangements of biological macromolecules. By archiving this data, the PDB enables researchers to study structure-function relationships and supports computational research in drug discovery and structural modeling.

**172.** (A) Vertebrates and selected model organisms
The Ensembl Genome Browser predominantly focuses on the genomes of vertebrates and a select group of model organisms. It provides high-quality automated annotation and comparative genomics data for species like humans, mice, and zebrafish, as well as agriculturally important livestock. This focus supports research into vertebrate evolution, gene regulation, and health by providing a standardized and comprehensive genomic resource.

**173.** (C) OR
In the Entrez retrieval system, the Boolean logic operator OR is used to expand a search by retrieving records that contain either one or both of the specified search terms. This is particularly useful when searching for synonyms, related concepts, or genes that may have multiple names, ensuring that the researcher captures a broader and more complete set of relevant biological records.

**174.** (A) A unique sequence identifier and optional description
In a standard FASTA file, the character ">" is immediately followed by a unique sequence identifier and an optional description on the same line. This header line provides essential metadata about the sequence that follows, such as the accession number and the name of the organism. Software tools use this identifier to distinguish between different records within a multi-sequence FASTA file.

**175.** (B) ExPASy Proteomics Server
The ExPASy Proteomics Server is broadly recognized for providing a comprehensive suite of protein analysis programs. These include tools for calculating isoelectric points, predicting post-translational modifications, and performing peptide mass fingerprinting for protein identification. Maintained by the Swiss Institute of Bioinformatics, ExPASy is a fundamental resource for researchers working in proteomics, providing reliable computational methods for protein functional analysis.

**176.** (C) Needleman-Wunsch
The Needleman-Wunsch algorithm is the standard dynamic programming method used to find the mathematically optimal global alignment across the entire lengths of two sequences. By evaluating all possible alignments and maximizing the overall similarity score, it provides a comprehensive comparison of two sequences. This algorithm is particularly useful for studying sequences that are suspected to be homologous over their entire length.

**177.** (D) 85%
A PAM matrix is derived by analyzing groups of protein sequences that share a high level of global identity, specifically at least 85%. This high threshold ensures that the observed amino acid substitutions are the result of direct mutations rather than multiple hits or complex evolutionary events. These initial observations are then mathematically extrapolated to create matrices suitable for more divergent sequences.

**178.** (B) The physical size of the search database
In a BLAST search, the E-value is directly proportional to the physical size of the search database. As the database grows larger, the number of alignments expected to occur by pure random chance also increases, leading to higher E-values for the same alignment score. Therefore, interpreting the statistical significance of a match requires considering the context of the total search space.

**179.** (B) tblastn
The tblastn program is the most appropriate BLAST variant for comparing a protein query against an unannotated whole-genome sequence. It translates the entire nucleotide database in all six reading frames in real-time and compares the protein query against these translations. This allows researchers to identify potential coding regions and homologous genes within raw genomic data that has not yet been annotated.

**180.** (A) Compute pairwise distance matrix → Construct guide tree → Perform progressive multiple alignment
The ClustalW progressive multiple alignment algorithm follows a specific three-step operational sequence. First, it computes a pairwise distance matrix by comparing all pairs of sequences in the dataset. Second, it constructs a guide tree based on these distances to determine the alignment

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that can fit. Predicting which specific 9-mer or 10-mer sequences will bind is a key task in immunoinformatics.

**217.** (A) Feature extraction / Representation learning
The capability of a deep learning model to automatically identify relevant features from raw data, such as DNA sequences, without manual human curation is termed feature extraction or representation learning. This allows the algorithm to discover complex patterns and relationships that might be non-obvious to researchers. This automated process is fundamental to the high performance of modern AI in genomics.

**218.** (A) Co-evolutionary distance maps between residue pairs
AlphaFold utilizes multiple sequence alignments to infer co-evolutionary distance maps between residue pairs as critical structural constraints. By analyzing how mutations in one position are compensated by mutations in another across related sequences, the model can predict which amino acids are spatially close in the folded protein. These distance maps serve as the foundation for constructing a highly accurate 3D model.

**219.** (A) Predictive and preventive discipline
The application of omics data integration in precision livestock farming is aimed at transitioning veterinary medicine from a reactive discipline to a predictive and preventive one. By identifying animals at high risk for disease before clinical symptoms appear, farmers and veterinarians can implement targeted interventions. This proactive approach improves animal welfare, increases production efficiency, and reduces the economic impact of disease.

**220.** (A) P-4, Q-1, R-2, S-3
Different AI and machine learning algorithms are suited for specific bioinformatics tasks. Random Forest provides robust ensemble classification for polygenic traits. Hidden Markov Models are widely used for sequence profile scanning and multiple alignment. Support Vector Machines excel at high-dimensional omics classification using hyperplanes. Artificial Neural Networks are applied to non-linear deep feature extraction, such as in transcriptomics and structural prediction.

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**221.** (A) Transfer RNA (tRNA)
Transfer RNA serves as the direct physical intermediary that links nucleotide codons in the mRNA to specific amino acids during the process of translation. Each tRNA molecule has an anticodon that recognizes a specific codon and carries the corresponding amino acid to the ribosome. This adapter function is essential for accurately translating the genetic code into a functional protein sequence.

**222.** (B) GenBank to MMDB (NCBI's structural database)
The NCBI Entrez system natively cross-links records, allowing a query for a gene sequence in GenBank to lead directly to its corresponding 3D structure in the Molecular Modeling Database. These links provide researchers with a seamless transition between sequence and structural information. This integration is essential for understanding the structural basis of gene function and for conducting molecular modeling studies.

**223.** (A) Nightly automated data exchange protocols
The global synchronization of sequence records between GenBank, EMBL, and DDBJ is ensured by nightly automated data exchange protocols. Under the INSDC agreement, each member database mirrors new and updated submissions from the others every 24 hours. This coordinated process ensures that the most current sequence information is available worldwide, regardless of which primary database received the original sequence submission.

**224.** (A) FSSP (DALI database)
FSSP is a structural database that categorizes protein domains by utilizing the DALI algorithm, which is a fully automated computational method for structure comparison. Unlike SCOP and CATH, which incorporate varying degrees of manual expert curation, FSSP relies entirely on the algorithm to detect structural similarities. This provides a purely objective, calculation-based view of structural relationships within the Protein Data Bank.

**225.** (A) Sequin / $tbl_2asn$
A researcher wishing to submit an exceptionally long or complex annotated sequence, such as a complete bacterial genome, to GenBank should use Sequin or the command-line tool $tbl_2asn$. These tools are designed to handle large-scale

descriptors. Because 3D descriptors are evaluated on a spatial grid, the number of variables often exceeds the number of molecules. PLS effectively reduces this dimensionality, allowing for the construction of stable and predictive models that correlate spatial interaction fields with biological activity.

**244.** (A) Hydropathy / Hydrophilicity.
To be identified by antibodies, B-cell epitopes must be surface-exposed and physically accessible. Predictive scales for hydropathy or hydrophilicity are critical for assessing this accessibility. Highly hydrophilic regions are likely to be found on the exterior of a folded protein, where they can interact with the aqueous environment and the immune system. Identifying these regions is key to predicting immunogenic B-cell epitopes.

**245.** (A) The cultivation of dangerous, fastidious pathogens in laboratory environments.
Reverse vaccinology dramatically reduces vaccine discovery time by bypassing the need to cultivate dangerous or fastidious pathogens in the laboratory. Instead, researchers use genomic data and bioinformatics tools to identify potential antigens in silico. This approach is safer, faster, and allows for the targeting of proteins that are not expressed under standard laboratory conditions, facilitating vaccine development for diverse organisms.

**246.** (A) The strength or importance of connections between node layers.
In Artificial Neural Networks, synaptic weights are computational parameters that represent the strength or importance of the connections between different node layers. During the training process, the network adjusts these weights to minimize prediction error. Higher weights indicate a stronger influence of a particular input on the final output. This weighting mechanism is essential for the network's ability to learn patterns.

**247.** (A) Kernels.
Support Vector Machines utilize specific functions, such as linear, polynomial, or radial basis functions, to handle non-linear data classifications. These functions are generically termed kernels. By applying the 'kernel trick,' the algorithm maps data into a higher-dimensional space where a linear separating hyperplane can be efficiently found. This makes SVMs powerful tools for complex classification tasks in biological sequence analysis.

**248.** (A) Force field
In molecular dynamics simulations, the set of mathematical functions and parameters used to describe the potential energy of a molecular system is termed a force field. It includes terms for bond stretching, angle bending, and non-bonded interactions like electrostatics and van der Waals forces. The accuracy of a simulation depends heavily on the quality and appropriateness of the chosen potential energy functions.

**249.** (A) Machine learning and Artificial Intelligence algorithms.
Precision livestock farming integrates massive, complex datasets from real-time sensors with genomic information. The primary analytical tool applied to interpret this data is machine learning and artificial intelligence algorithms. These tools can identify patterns and predict animal health or production outcomes by processing heterogeneous data sources simultaneously. This enables farmers to implement proactive management strategies based on sophisticated, data-driven insights.

**250.** (A) I, II, III and V
Reverse vaccinology is a modern method that requires whole genome sequences to mathematically identify potential antigens. It is particularly effective at finding non-structural antigens and relies on algorithms that predict surface localization. While it accelerates discovery, it does not bypass the need for ultimate in vivo testing to ensure safety and efficacy. Thus, statements I, II, III, and V are correctly stated.

**251.** (A) Alternative splicing
Alternative splicing is the biological mechanism that enables a single eukaryotic gene to encode multiple distinct protein isoforms. During this process, different combinations of exons from the same pre-mRNA are joined together, while others are removed as introns. This increases the functional diversity of the proteome without expanding the genome, allowing cells to produce specialized proteins for different developmental stages.

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While computationally efficient, this approach means that errors made early in the process cannot be corrected, potentially leading to suboptimal results.

**235.** (A) A point of sequence mutation or speciation, implying an ancestor.
In a phylogenetic tree, a node represents a point of evolutionary divergence, such as a speciation event or a sequence mutation, implying a common ancestor for the branches that originate from it. Internal nodes represent ancestral lineages, while terminal nodes, or leaves, represent the extant taxa being studied. The arrangement of nodes and branches defines the topology of the evolutionary tree.

**236.** (A) Rooting the tree using an outgroup.
The Neighbor-Joining method naturally constructs an unrooted phylogenetic tree, which shows relationships but lacks directionality. To provide evolutionary context and identify the order of divergence, a researcher must computationally root the tree using an outgroup. An outgroup is a taxon known to have diverged earlier than the group being studied, establishing the ancestral state and the flow of evolutionary time.

**237.** (A) Two, each present in at least two different taxa.
In Maximum Parsimony, an informative site is defined as a position in a sequence alignment that contains at least two distinct character states, each present in at least two different taxa. These sites provide the necessary information to distinguish between different tree topologies. Sites that do not meet these criteria are considered uninformative because they require the same number of changes.

**238.** (A) Annotating highly conserved orthologous genes in closely related species.
Homology-based gene prediction is highly effective when annotating conserved orthologous genes in closely related species. By leveraging the sequence similarity to known, well-characterized genes, these tools can accurately identify exon boundaries and functional domains. This evolutionary approach is generally more reliable than ab initio methods when a high-quality reference transcript or a closely related genome is available for search.

**239.** (A) $\log P$
The molecular property that evaluates a molecule's lipophilicity is $\log P$, which is the logarithm of the partition coefficient between octanol and water. As specified in Lipinski's Rule of Five, $\log P$ is crucial for determining a compound's ability to penetrate lipid bilayers. Proper lipophilicity is essential for oral bioavailability, as a drug must be soluble in water but also oily enough to cross membranes.

**240.** (A) I, II and IV
Molecular descriptors in QSAR vary by dimensionality. 1D descriptors include global properties like molecular weight, while 2D descriptors involve connectivity indices. 3D descriptors evaluate spatial fields, such as steric and electrostatic interactions. However, 3D descriptors are not independent of conformation; they are highly dependent on the three-dimensional arrangement and alignment of the molecule. Thus, statements I, II, and IV are correct.

**241.** (A) Functional groups necessary for receptor recognition.
Pharmacophore modeling relies on identifying the spatial arrangement of specific functional groups that are necessary for a molecule to be recognized by a target receptor. These features, such as hydrogen bond donors, acceptors, and aromatic rings, are placed in a three-dimensional framework. By understanding this required pattern, researchers can design or screen for new compounds that possess the same essential structural characteristics.

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**242.** (A) Rapidly evaluate and rank a massive library of compounds against a structural target.
In Computer-Aided Drug Design, virtual screening leverages docking algorithms to rapidly evaluate and rank a massive library of chemical compounds against a specific structural target. This high-throughput computational approach identifies the molecules most likely to bind with high affinity, allowing researchers to prioritize specific candidates for experimental testing. This significantly reduces the time and cost associated with drug discovery.

**243.** (A) 3D-QSAR (e.g., CoMFA)
Partial Least Squares regression is extensively utilized in 3D-QSAR modeling, such as CoMFA, to handle the challenges of highly correlated

clade. Both refer to a grouping that includes a common ancestor and all of its descendants. The concept of a clade is the basis for modern biological classification systems, as it represents a natural evolutionary unit. Identifying clades is essential for reconstructing the tree of life and understanding evolutionary history.

**208.** (A) Neighbor-Joining
Neighbor-Joining is a distance-based phylogenetic algorithm that explicitly constructs an unrooted tree and relaxes the strict molecular clock assumption. Unlike UPGMA, which assumes equal rates of evolution across all branches, Neighbor-Joining allows for varying rates of change. This makes it a more robust and widely applicable method for analyzing diverse biological sequences and reconstructing evolutionary relationships with high efficiency.

**209.** (B) Maximum Parsimony
Long-branch attraction is a statistical artifact that predominantly affects the Maximum Parsimony method of phylogenetic reconstruction. It occurs when unrelated taxa with rapidly evolving sequences are mistakenly grouped together because they coincidentally share more random similarities with each other than with their true relatives. This artifact can lead to the inference of incorrect tree topologies, particularly when evolutionary rates vary significantly.

**210.** (A) II, III, I, IV
Ab initio gene prediction algorithms like GENSCAN follow a progressive sequence of logical steps. The process begins with identifying potential open reading frames. Next, start and stop codons are localized to define the boundaries. Splice site prediction is then performed to identify donor and acceptor sites for introns. Finally, the predicted exons are assembled into a coherent gene model for annotation.

**211.** (C) 10
According to Lipinski's Rule of Five, poor oral absorption is more likely if a chemical compound contains more than 10 hydrogen bond acceptors. This count is determined by the total number of nitrogen and oxygen atoms in the molecule. Maintaining a reasonable number of hydrogen bond acceptors is important for balancing the molecule's polarity and ensuring it can cross biological membranes.

**212.** (A) 3D-QSAR (e.g., CoMFA)
The methodology that maps a theoretical grid around aligned ligand molecules to compute steric and electrostatic interaction fields is known as 3D-QSAR, with CoMFA being a prominent example. By evaluating these fields at every grid point, the model identifies specific regions where changes in molecular volume or charge correlate with biological activity, providing detailed guidance for optimizing drug potency.

**213.** (C) Force-field-based scoring functions
Molecular docking scoring functions that rely fundamentally on basic physical principles, such as Coulombic potentials for electrostatics and Lennard-Jones potentials for van der Waals forces, are categorized as force-field-based scoring functions. These functions estimate the total binding energy by summing the individual physical interaction terms. They provide a physically grounded approach to evaluating the stability and affinity of protein-ligand complexes.

**214.** (B) Steric parameter
In QSAR modeling, a steric parameter is the specific descriptor type that directly addresses the three-dimensional volumetric shape and bulkiness of a drug molecule. These parameters, such as Taft's steric constant or Verloop's STERIMOL values, quantify the spatial requirements of molecular groups. Understanding steric effects is crucial for predicting how well a molecule will fit into a target's binding pocket.

**215.** (A) Complete genomic sequences of the target pathogen
Reverse vaccinology identifies candidate protective antigens by computationally analyzing the complete genomic sequences of a target pathogen. Instead of growing the pathogen in the lab, researchers use bioinformatics tools to screen the entire genome for genes that likely encode surface-exposed or secreted proteins. This in silico approach enables the rapid discovery of potential vaccine targets, even for unculturable organisms.

**216.** (B) 8-11 residues
T-cell epitopes presented by Major Histocompatibility Complex Class I molecules are generally short peptide fragments, typically between 8 and 11 residues in length. The binding pocket of MHC Class I molecules is closed at both ends, which restricts the size of the peptides

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**252.** (B) GenBank / nr (non-redundant database)
A researcher who discovers a novel enzyme in an extreme thermophile should immediately query the GenBank non-redundant database. The 'nr' database contains all publicly available protein and translated nucleotide sequences. Performing a search against this comprehensive repository is the standard procedure to determine if a sequence is truly novel or if related enzymes have already been sequenced by other researchers.

**253.** (A) Highly curated evolutionary superfamilies.
The PIR-PSD distinguishes itself from the TrEMBL database by focusing on the classification of proteins into highly curated evolutionary superfamilies. While TrEMBL provides automated translations, PIR emphasizes the evolutionary relationships and functional context of protein sequences. This classification system helps researchers understand the origin and diversity of protein families, providing a deeper biological understanding than simple sequence archiving alone.

**254.** (A) CATH is partially automated using structural algorithms; SCOP relies more heavily on expert manual curation.
A fundamental difference between CATH and SCOP structural classifications lies in their methodology. CATH is partially automated, utilizing sophisticated structural algorithms like DALI and SSAP to identify relationships, which are then reviewed by curators. In contrast, SCOP relies more heavily on expert manual curation and visual inspection of evolutionary relationships. Both provide essential but slightly different perspectives on the protein structural landscape.

**255.** (A) RefSeq provides a single, non-redundant, stable reference record for each biological molecule.
The primary advantage of the RefSeq database over GenBank's standard repository is that RefSeq provides a single, non-redundant, and stable reference record for each biological molecule. While GenBank archives all submissions, RefSeq curators synthesize this data into a high-quality standard. These reference sequences are essential for ensuring consistency across genomic studies, providing a reliable baseline for annotation and variant analysis.

**256.** (A) Marker-Assisted Selection (MAS) and genomic breeding.
AnimalQTLdb is specifically structured to support marker-assisted selection and genomic breeding in agriculture. By archiving the associations between genetic markers and important phenotypic traits, the database provides the evidence needed to develop predictive breeding models. This allows animal scientists to select for superior traits, such as increased growth or disease resistance, more accurately and efficiently, leading to significant improvements in production.

**257.** (A) Records containing either $BRCA_1$ or $BRCA_2$, strictly within humans.
In an Entrez search, the query '($BRCA_1$ OR $BRCA_2$) AND human' will definitively yield records containing either the $BRCA_1$ gene or the $BRCA_2$ gene, but only if they are also associated with humans. The parentheses ensure that the OR operator is evaluated first, grouping the two genes together, while the AND operator restricts the entire set of results to human-specific genomic records.

**258.** (A) EMBL.
The EMBL sequence format is characterized by its use of specific two-letter keyword tags at the start of each line, such as ID for identification, AC for accession number, and SQ for sequence. This structured text format is designed for easy parsing by bioinformatics software. It provides a standardized way to store both sequence data and extensive biological metadata, ensuring high data interoperability.

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**259.** (A) Complex biological feature annotations (e.g., exon/intron boundaries) within the sequence file itself.
A key limitation of the basic FASTA format is its inability to natively support complex biological feature annotations within the sequence file itself. While it excels at storing raw sequence data with a simple header, it cannot represent detailed information like exon-intron boundaries, protein domains, or secondary structure. For such detailed data, more complex formats like GenBank flat files are required.

**260.** (A) Both A and R are true and R is the correct explanation of A.
The Smith-Waterman algorithm is computationally more demanding than heuristic

algorithms like BLAST because it explores the entire dynamic programming matrix to guarantee mathematical optimality. In contrast, BLAST restricts the search space by using short word seeds to find regions of potential similarity. While Smith-Waterman is more sensitive, BLAST's heuristic approach is essential for searching massive volumes of modern data.

**261.** (B) The match is statistically highly significant and extremely unlikely to be due to chance.
A BLAST E-value of $1 \times 10^{-50}$ strongly implies that the sequence match is biologically significant and evolutionary homology is highly probable. An E-value this low indicates that the number of such matches expected to occur by chance is virtually zero. This provides a high degree of confidence that the query and database sequences share a common evolutionary ancestor and potentially similar biological functions.

**262.** (A) Traceback.
In dynamic programming sequence alignments, the traceback step is the concept that ensures the algorithm remembers the path yielding the highest score to generate the final alignment string. After the scoring matrix is filled, the algorithm starts from the optimal cell and follows the pointers back to the beginning. This process reconstructs the sequence of matches, mismatches, and gaps that constitute the alignment result.

**263.** (A) The substitution occurs more frequently than expected by chance.
A positive score in a BLOSUM matrix indicates that a specific amino acid substitution occurs more frequently in nature than would be expected by chance. These scores are derived from log-odds ratios based on observed substitution frequencies in conserved protein blocks. A positive value suggests that the two amino acids are chemically or structurally similar enough to be evolutionarily interchangeable without disrupting biological function.

**264.** (A) Hidden Markov Models (HMMs) via the mBed algorithm.
Clustal Omega replaced ClustalW by introducing an algorithm that utilizes Hidden Markov Models and the mBed strategy. This approach allows it to handle hundreds of thousands of sequences efficiently, which was impossible for older progressive methods. By using HMMs for profile alignment and mBed for rapid clustering, Clustal Omega provides high-speed, high-quality multiple sequence alignments for the massive datasets of today.

**265.** (A) Pairwise alignments.
T-Coffee is a consistency-based multiple sequence alignment algorithm that attempts to maximize agreement between the final multiple alignment and a pre-computed library of pairwise alignments. This library includes both global and local alignments for every pair of sequences in the set. By considering all pairwise information simultaneously, T-Coffee produces more accurate and robust results, especially for divergent protein families where methods fail.

**266.** (A) The magnitude of evolutionary change or elapsed time.
A phylogram is explicitly distinguished from a cladogram because its branch lengths directly represent the magnitude of evolutionary change or elapsed time. In a phylogram, the horizontal distance of a branch is proportional to the number of genetic mutations that have occurred along that lineage. This provides a more detailed view of the evolutionary process than a cladogram, which only shows branching relationships.

**267.** (A) Homoplasy.
Homoplasy is a phenomenon that introduces noise into phylogenetic analysis by creating identical character states in separate lineages that are not due to shared ancestry. This can result from convergent evolution, parallel evolution, or evolutionary reversals. Because homoplastic traits mimic homology, they can lead to the construction of incorrect evolutionary trees. Distinguishing between homology and homoplasy is a challenge in systematics.

**268.** (A) Mathematical model of nucleotide substitution (e.g., General Time Reversible).
Maximum Likelihood phylogenetic analysis relies heavily on selecting an appropriate mathematical model of nucleotide or amino acid substitution, such as the General Time Reversible model. These models provide the probabilistic framework for calculating the likelihood of the observed sequence data. By accounting for varying substitution rates and base frequencies, the chosen model significantly influences the accuracy of the inferred evolutionary topology.

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**269.** (A) Introns
Eukaryotic gene prediction is substantially more complex than prokaryotic prediction primarily due to the presence of introns. These non-coding regions interrupt the protein-coding exons, requiring algorithms to accurately identify splice sites to reconstruct the correct mRNA sequence. Identifying short exons within massive introns is a significant computational challenge, making eukaryotic gene finding far more difficult than predicting continuous coding regions.

**270.** (A) Coding sequences (exons) are generally more highly conserved than non-coding sequences (introns).
Homology-based gene prediction is highly reliable because it leverages the evolutionary principle that coding sequences, or exons, are generally more highly conserved than non-coding sequences, or introns. By comparing a new genome to known genes from related species, researchers can identify these conserved regions. This homology serves as strong evidence for gene locations and exon boundaries, facilitating the accurate genomic annotation.

**271.** (A) One
According to Lipinski's Rule of Five, an orally active drug candidate generally violates no more than one of the specified criteria to maintain a favorable pharmacokinetic profile. While meeting all four rules is ideal, a single violation does not automatically disqualify a compound. However, molecules that violate two or more rules are significantly more likely to suffer from poor absorption and low oral bioavailability.

**272.** (A) Molecular Dynamics (MD) simulations.
In Computer-Aided Drug Design, a common technique to refine a docked pose and simulate real physiological interactions over time is Molecular Dynamics simulation. MD evaluates the physical movements of atoms and molecules by numerically solving Newton's equations of motion. This allows researchers to study the stability of a protein-ligand complex and the impact of solvent and temperature on the binding process.

**273.** (A) It incorporates a local conformational search whose improved pose is passed directly back to the genome.
The Lamarckian aspect of the genetic algorithm in AutoDock modifies the standard approach by incorporating a local conformational search whose improved pose is passed directly back to the genetic record, or 'genome,' of the individual. This hybrid strategy allows the algorithm to quickly find the local energy minimum for each pose, combining global search efficiency with local optimization for more accurate results.

**274.** (A) Overfitting
In QSAR modeling, validating the model purely on the training set can lead to the significant statistical error known as overfitting. Overfitting occurs when a model becomes so complex that it captures the noise in the training data rather than the underlying relationship. As a result, the model performs perfectly on the training set but fails to accurately predict the activity of new, unseen compounds.

**275.** (A) Surface-exposed or secreted.
Reverse vaccinology heavily relies on specific predictive algorithms to ensure that the selected antigen target is localized on the surface of the pathogen or secreted into the environment. These regions are more likely to be accessible to the host's immune system, specifically to antibodies and B-cells. Identifying these targets in silico is a crucial step for designing effective vaccines that trigger protective immunity.

**276.** (A) Linear, because they are presented as digested peptides on MHC molecules.
T-cell epitopes are generally linear in nature because they are presented to the immune system as short, digested peptide fragments bound to MHC molecules. Unlike B-cell epitopes, which can recognize the 3D shape of a folded protein, T-cell receptors only recognize peptides that have been processed and displayed on the surface of host cells. This linear presentation is fundamental to immunity.

**277.** (A) Understanding exactly how the network combines input features to reach its final classification.
The 'black box' problem in Artificial Neural Networks refers to the inherent difficulty in understanding exactly how the network combines complex input features to reach its final classification. Because information is processed through multiple hidden layers with non-linear connections, the internal reasoning of the model is not easily interpretable by humans. This lack

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(A) Methylate specific DNA cytosine residues
(B) Evict or reposition nucleosomes
(C) Acetylate various histone tail residues
(D) Ligate lagging strand Okazaki fragments

2002
**11.** Match the items in List I with the items in List II:
List I: (Chromatin Component)
P. Linker DNA
Q. $H_1$ Histone
R. Scaffold proteins
S. Telomeres
List II: (Function/Feature)
1. Seals the nucleosome
2. Forms chromosomal loops
3. Protects chromosome ends
4. Connects adjacent nucleosomes
(A) P-I, Q-IV, R-II, S-III
(B) P-IV, Q-I, R-II, S-III
(C) P-IV, Q-I, R-III, S-II
(D) P-II, Q-IV, R-I, S-III

2012
**12.** Scaffold Attachment Regions (SARs) are predominantly rich in which nucleotides?
(A) GC
(B) Modified uracils
(C) AT
(D) Methylated cytosines

2024
**13.** Single-copy eukaryotic DNA sequences are primarily responsible for encoding:
(A) Ribosomal RNA
(B) Transfer RNA
(C) Centromeric structural elements
(D) Structural and functional proteins

2009
**14.** Which sequence class represents the highest proportion of an average mammalian genome?
(A) Single-copy structural genes
(B) Highly repetitive DNA
(C) Moderately repetitive DNA
(D) Ribosomal DNA clusters

2001
**15.** The reassociation kinetics of DNA, often plotted as a Cot curve, show that highly repetitive DNA reanneals:
(A) At the slowest rate
(B) At the fastest rate
(C) Only at low temperatures
(D) Simultaneously with single-copy DNA

2006
**16.** Moderately repetitive DNA in eukaryotes generally includes which of the following?
(A) Exons of major metabolic enzymes
(B) Alpha satellite arrays
(C) Telomeric TTAGGG repeats
(D) Genes for rRNAs and tRNAs

2011
**17.** Consider the following statements regarding eukaryotic DNA classes:
I. Highly repetitive DNA reassociates fastest in Cot analysis.
II. Highly repetitive DNA is mostly non-coding.
III. SINEs belong to the moderately repetitive class.
IV. Satellite DNA forms a distinct band from the main band in density gradient centrifugation.
Which of the above statement(s) is/are correct?
(A) I, II and III
(B) I, III and IV
(C) I and IV
(D) I, II, III and IV

2025
**18.** Satellite DNA separates from bulk genomic DNA during density gradient centrifugation due to its distinct:
(A) Chromatin packaging state
(B) Exon-intron structure
(C) Base composition (GC content)
(D) Histone acetylation level

2021
**19.** Highly repetitive DNA is predominantly localized to which chromosomal regions?
(A) Telomeres and centromeres
(B) Enhancers and promoters
(C) Introns of housekeeping genes
(D) Subtelomeric coding domains

2018
**20.** Which class of DNA sequence possesses the highest genetic complexity?
(A) Alpha satellite DNA
(B) Minisatellites
(C) SINEs
(D) Single-copy DNA

2001
**21.** What is the correct sequence of repetitive DNA elements ordered from the shortest basic repeat unit length to the longest?
(A) Satellite → Minisatellite → Microsatellite
(B) Microsatellite → Satellite → Minisatellite
(C) Microsatellite → Minisatellite → Satellite

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topological arrangement. Proteins within the same fold have similar three-dimensional shapes, even if they lack significant sequence similarity or a clear evolutionary relationship. This level of classification helps scientists understand the fundamental building blocks and structural constraints that govern folding.

**287.** (A) Integrated comparative genomics tracks, displaying synteny and ortholog alignments.
A unique feature of the Ensembl Genome Browser that aids in evolutionary genomics is its integrated comparative genomics tracks. These tracks allow researchers to visualize synteny and ortholog alignments across distinct vertebrate species simultaneously. By displaying how gene order and sequence are conserved between organisms, Ensembl provides powerful insights into genome evolution, gene function, and structural changes since species diverged.

**288.** (A) An ATG start codon and an in-frame stop codon, exceeding a minimal length threshold.
The ORF Finder tool typically defines an Open Reading Frame as a sequence of DNA that begins with an ATG start codon and ends with an in-frame stop codon, provided it exceeds a minimal length threshold. Identifying these potential protein-coding segments is a basic step in gene prediction. By scanning all six reading frames, researchers can locate candidate genes for further analysis.

**289.** (A) The extreme bottom-right cell of the matrix.
In dynamic programming for global sequence alignment, the traceback step always originates from the extreme bottom-right cell of the scoring matrix. This cell represents the optimal alignment score for the entire length of both sequences. From this point, the algorithm follows the pointers back to the origin, reconstructing the exact sequence of matches, mismatches, and gaps that produce the best overall alignment result.

**290.** (A) An un-gapped local extension evaluates if a high-scoring segment pair (HSP) exists.
In the BLAST heuristic algorithm, if a query word scores above a specific threshold 'T' against a database word, it triggers an un-gapped local extension. The algorithm evaluates the sequences surrounding the match to see if a high-scoring segment pair exists. If the score continues to increase, the extension continues until it reaches a peak, effectively identifying regions of potential biological homology.

**291.** (B) Prevent statistically significant but biologically spurious hits.
The primary purpose of 'masking' low-complexity regions in a BLAST search, such as simple sequence repeats, is to prevent statistically significant but biologically spurious hits. These repetitive regions can often match each other by chance, leading to very low E-values that do not reflect true evolutionary homology. Masking these areas ensures that the search results are focused on more meaningful sequence similarities.

**292.** (A) Aligning two distinct dynamic programming matrices.
In multiple sequence alignment, a profile represents a probabilistic model of an aligned block of sequences. Aligning two profiles together is mathematically similar to aligning two distinct dynamic programming matrices. Instead of comparing individual residues, the algorithm compares probability distributions of amino acids at each position. This approach is highly sensitive and is used to detect distant evolutionary relationships between protein families.

**293.** (A) Analogs
Analogs are distinct, non-homologous proteins that have evolved entirely independently but possess the exact same biological function. This phenomenon is a result of convergent evolution, where different structural solutions arise to fulfill the same functional need. Unlike orthologs and paralogs, which share a common ancestor, analogs have different evolutionary origins, making them interesting subjects for studying structural biology and convergence.

**294.** (A) Highly divergent, accumulating numerous multiple hits at identical sites.
Maximum Parsimony is generally considered least reliable when analyzing sequences that are highly divergent. In these cases, numerous multiple mutations, or 'hits,' can accumulate at the same nucleotide positions. Parsimony has difficulty distinguishing between these complex evolutionary changes and simple shared derived characters. This often leads to artifacts like long-branch attraction, where unrelated rapidly evolving lineages are incorrectly grouped together.

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of transparency is a challenge in clinical decision-making.

**278.** (B) Principal Component Analysis (PCA).
Principal Component Analysis is an unsupervised machine learning technique primarily used to reduce the dimensionality of high-throughput omics data. By transforming the original variables into a new set of orthogonal principal components, PCA retains the most significant variance in the data while simplifying its structure. This allows researchers to visualize clusters, identify outliers, and detect patterns in complex gene expression datasets.

**279.** (A) Structure-based virtual screening and molecular docking.
AlphaFold significantly accelerates structural bioinformatics, and the process that most directly benefits from the influx of these predicted structures is structure-based virtual screening and molecular docking. By providing high-accuracy models for proteins that were previously uncharacterized, AlphaFold expands the range of targets available for drug discovery. Researchers can now use these models to screen for new inhibitors and study binding interactions.

**280.** (A) P-2, Q-1, R-4, S-3
Bioinformatics sequence formats are identified by their characteristic start symbols. The FASTA format uses a greater-than symbol, while FASTQ uses an '@' symbol to mark the beginning of a record. The GenBank format is identified by the keyword 'LOCUS' at the start of the first line. The EMBL format begins with the 'ID' tag. Recognizing these symbols is essential for sequence data parsing.

**281.** (A) Estimated Breeding Value (EBV).
Genomic selection in animal health utilizes genome-wide marker data to calculate the Estimated Breeding Value of an individual. This quantitative metric represents an animal's genetic merit for specific traits, allowing breeders to select the best parents for the next generation. By using genetic markers instead of just phenotypes, genomic selection increases the accuracy of these values and speeds up genetic improvement.

**282.** (A) Quaternary
The quaternary level of protein structure describes the arrangement of multiple distinct protein subunits into a multi-subunit functional complex. This level of organization involves non-covalent interactions and disulfide bonds that hold the individual polypeptide chains together. Understanding quaternary structure is essential for explaining the function of many complex proteins, such as hemoglobin or DNA polymerase, which require multiple subunits.

**283.** (A) Short nucleotide variations, primarily Single Nucleotide Polymorphisms.
The primary role of the dbSNP database maintained by the National Center for Biotechnology Information is to catalog short nucleotide variations, focusing mainly on Single Nucleotide Polymorphisms. It also includes information on small-scale insertions, deletions, and other variations. dbSNP is a fundamental resource for researchers studying genetic diversity, evolutionary biology, and the association between specific genetic variants and inherited diseases.

**284.** (A) InterPro
InterPro is a massive, internationally collaborative secondary database that integrates information on protein families, domains, and functionally important sites. By combining data from several member databases like PROSITE, Pfam, and SMART, InterPro provides a unified resource for protein sequence analysis and functional classification. It is a vital tool for the automated annotation of newly sequenced proteins and for understanding their evolutionary contexts.

NODIA

**285.** (A) Expert curators manually merge reports of the identical protein into a single, comprehensive record.
In the Swiss-Prot database, non-redundancy is ensured by expert curators who manually merge multiple reports of the identical protein into a single, comprehensive record. Instead of having separate entries for each time a protein is sequenced, the curators synthesize all available information, including sequence variations and functional data, into one definitive entry. This meticulous process ensures high data quality and accuracy.

**286.** (A) Major secondary structures in the same topological arrangement.
In the SCOP classification system, a 'Fold' is defined by groups of proteins that share the same major secondary structures in the same

# CHAPTER 09

# Animal Genomics and Genome Editing Technology

2001

**1.** What is the fundamental repeating unit of eukaryotic chromatin?
(A) Nucleosome
(B) Chromatosome
(C) Solenoid
(D) Centromere

2024

**2.** Which histone protein is NOT a part of the core histone octamer?
(A) $H_2A$
(B) $H_2B$
(C) $H_1$
(D) $H_4$

2010

**3.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.
**Assertion (A):** Euchromatin represents transcriptionally active regions of the genome.
**Reason (R):** Euchromatin is less densely packed, allowing transcription factors to access the DNA.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2025

**4.** Approximately how many base pairs of DNA wrap around the histone octamer in a core nucleosome?
(A) 166 bp
(B) 200 bp
(C) 250 bp
(D) 147 bp

2003

**5.** Which level of hierarchical genome packaging involves a 30 nm fiber?
(A) Naked DNA
(B) Solenoid structure
(C) Looped domains
(D) Metaphase chromosome

2005

**6.** Heterochromatin is typically characterized by which of the following epigenetic marks?
(A) High levels of histone acetylation
(B) Unmethylated CpG islands
(C) Histone $H_3K_9$ methylation
(D) RNA polymerase II binding

2010

**7.** Consider the following statements regarding nucleosome assembly:
I. Assembly requires histone chaperones like CAF-1.
II. The $H_3$-$H_4$ tetramer binds DNA before $H_2A$-$H_2B$ dimers.
III. Nucleosomes exclusively form on GC-rich regions.
Which of the above statement(s) is/are correct?
(A) I and II
(B) I and III
(C) I
(D) III

2016

**8.** The zig-zag model is an alternative structural hypothesis for which chromatin conformation?
(A) 30 nm fiber
(B) 10 nm fiber
(C) 300 nm loops
(D) 700 nm chromatid

2021

**9.** In standard eukaryotic DNA, which fraction is transcriptionally silent and replicates late in the S phase?
(A) Facultative euchromatin
(B) Constitutive heterochromatin
(C) Telomerase RNA
(D) Active promoter regions

2015

**10.** Chromatin remodeling complexes utilizing ATP hydrolysis primarily function to:

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**295.** (A) Bootstrapping.
Bootstrapping is a statistical technique used to evaluate the robustness of a phylogenetic tree. It involves randomly resampling columns from the original sequence alignment to create multiple pseudo-replicate datasets. For each replicate, a new tree is constructed. The bootstrap value for a specific branch represents the percentage of replicate trees that contain that grouping, providing a measure of how strongly the data supports that relationship.

**296.** (A) Short initial and terminal exons bounded by massive introns.
Eukaryotic ab initio gene prediction algorithms primarily struggle with identifying short initial and terminal exons that are surrounded by massive introns. Because these exons are small and separated by vast non-coding regions, their statistical signals are easily lost in the genomic noise. Accurately predicting the start and end of these complex gene models remains one of the most significant challenges in computational genomics.

**297.** (A) The compound severely violates Lipinski's Rule of Five and is highly unlikely to be orally bioavailable.
A compound with a molecular weight of 750 Da, 12 hydrogen bond donors, and a $\log P$ of 8 severely violates several criteria of Lipinski's Rule of Five. Specifically, it exceeds the thresholds for weight, donors, and lipophilicity. Consequently, the most appropriate conclusion is that the compound is highly unlikely to be orally bioavailable, as it would probably suffer from poor solubility and membrane permeability.

**298.** (B) Six
In molecular docking, a purely rigid search space requires evaluating a maximum of six degrees of freedom. These consist of three translational degrees of freedom, which describe the movement of the molecule along the $x$, $y$, and $z$ axes, and three rotational degrees of freedom, which describe its orientation. By keeping the internal bonds fixed, the algorithm only needs to find the optimal position and rotation.

**299.** (A) They are highly surface-exposed, readily accessible to host antibodies.
Reverse vaccinology frequently screens bacterial outer membrane proteins because they are highly surface-exposed and readily accessible to host antibodies. As the primary point of contact between the pathogen and the host's immune system, OMPs are excellent candidate antigens for vaccine development. Their location on the exterior of the bacterium makes them prime targets for neutralization and for triggering a protective immune response against pathogens.

**300.** (C) Highly accurate, predictive genomic selection of traits.
In veterinary bioinformatics, integrating high-throughput genomic marker data with machine learning, such as Random Forests, fundamentally shifts breeding practices. Instead of relying solely on phenotypic evaluation, which can be slow and imprecise, breeders can now use highly accurate, predictive genomic selection of traits. This allows for the identification of superior animals early in life, significantly accelerating genetic progress and improving animal health outcomes.

**********

NODIA

(D) Minisatellite → Microsatellite → Satellite

2024
**22.** Alu elements in the human genome are a classic example of:
(A) Highly repetitive tandemly arrayed repeats
(B) Single-copy protein-coding functional genes
(C) Moderately repetitive interspersed sequences
(D) Telomeric terminal hexanucleotide repeats

2001
**23.** Functional pseudogenes typically fall under which classification of genomic DNA?
(A) Highly repetitive
(B) Moderately repetitive
(C) Tandem repeats
(D) Single-copy

2013
**24.** Genomic regions containing tandemly repeated sequences spanning hundreds of kilobases are primarily classified as:
(A) Satellite DNA
(B) MicroRNAs
(C) Macroadenomas
(D) Single-copy DNA

2009
**25.** Long Interspersed Nuclear Elements (LINEs) are structurally classified as:
(A) DNA transposons
(B) Non-LTR retrotransposons
(C) LTR retrotransposons
(D) Plasmids

2005
**26.** Which intrinsic enzymes are encoded by functional LINEs to facilitate their autonomous mobilization?
(A) DNA polymerase and ligase enzymes
(B) Reverse transcriptase and endonuclease
(C) RNA polymerase and helicase enzymes
(D) Integrase and transposase enzymes

2009
**27.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.
**Assertion (A):** Short Interspersed Nuclear Elements (SINEs) are considered non-autonomous retrotransposons.
**Reason (R):** SINEs do NOT encode their own reverse transcriptase or endonuclease enzymes.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2010
**28.** SINEs are typically transcribed by which host enzyme?
(A) RNA polymerase I
(B) RNA polymerase II
(C) RNA polymerase III
(D) Reverse transcriptase

2018
**29.** Consider the following statements concerning interspersed repeats:
I. LINE-1 elements comprise about 20% of the mammalian genome.
II. SINEs depend on LINE-encoded machinery for retrotransposition.
III. Interspersed retrotransposons transpose via a "copy-and-paste" mechanism.
IV. Target Site Duplications (TSDs) flank newly inserted LINEs.
V. Alu elements originated from the 7SL RNA gene.
Which of the above statement(s) is/are correct?
(A) I, II, III and V
(B) I, III, IV and V
(C) I, II, IV and V
(D) All of the above

2004
**30.** Which cellular RNA represents the evolutionary ancestor of the Alu element?
(A) 16S rRNA
(B) tRNA-Lys
(C) 7SL RNA
(D) $U_1$ snRNA

2003
**31.** Retrotransposon insertion creates short direct repeats at the integration locus known as:
(A) Long Terminal Repeats (LTRs)
(B) Target Site Duplications (TSDs)
(C) Inverted Repeats (IRs)
(D) Variable Number Tandem Repeats (VNTRs)

2013
**32.** In livestock genomes, SINEs are primarily derived from which host sequence?
(A) rRNAs
(B) Spliceosomal snRNAs
(C) Mitochondrial loop DNA
(D) tRNAs

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2001

**33.** Match the items in List I with the items in List II:

List I: (Transposable Element)

P. LINE-1

Q. Alu element

R. LTR retrotransposon

S. DNA transposon

List II: (Characteristic)

1. Relies on reverse transcriptase from other elements
2. Mobilizes via "cut-and-paste"
3. Autonomous non-LTR element
4. Structurally mimics retroviruses

(A) P-III, Q-I, R-IV, S-II

(B) P-I, Q-III, R-II, S-IV

(C) P-III, Q-I, R-II, S-IV

(D) P-II, Q-IV, R-III, S-I

2010

**34.** The predominant mechanism limiting LINE retrotransposition in mature somatic cells is:

(A) Alternative splicing

(B) Telomere shortening

(C) DNA methylation

(D) Exonuclease degradation

2002

**35.** Bovine Repetitive Element 1 (Bov-B) is a prominent interspersed repeat in cattle that is historically characterized by:

(A) Horizontal transfer from squamate reptiles

(B) DNA transposition via a cut-and-paste mechanism

(C) Exclusive localization to centromeric regions

(D) Total absence of target site duplication markers

2002

**36.** An intact, full-length mammalian LINE-1 element is approximately how long?

(A) 300 bp

(B) 1.5 kb

(C) 6.0 kb

(D) 12.0 kb

2004

**37.** What is the standard diploid chromosome number ($2n$) for Bos taurus (cattle)?

(A) 48

(B) 54

(C) 60

(D) 78

2007

**38.** In traditional karyotyping, cell division is halted to visualize chromosomes using which chemical?

(A) Phytohemagglutinin

(B) Ethidium bromide

(C) Colchicine

(D) Giemsa

2002

**39.** Consider the following statements regarding diploid chromosome numbers ($2n$) in farm animals:

I. The domestic sheep (Ovis aries) has $2n = 54$.

II. The domestic goat (Capra hircus) has $2n = 60$.

III. The domestic pig (Sus scrofa) has $2n = 38$.

Which of the above statement(s) is/are correct?

(A) I, II and III

(B) I and III

(C) I

(D) III

NODIA

2004

**40.** Chromosomes are optimally condensed and photographed for karyotyping during which phase of mitosis?

(A) Prophase

(B) Metaphase

(C) Anaphase

(D) Telophase

2021

**41.** Arrange the following livestock species in correct order based on their standard diploid chromosome number (lowest to highest):

I. Swine (Sus scrofa)

II. River buffalo (Bubalus bubalis)

III. Cattle (Bos taurus)

IV. Chicken (Gallus gallus)

(A) I, II, III, IV

(B) II, I, III, IV

(C) IV, III, II, I

(D) I, III, II, IV

2001

**42.** What is the standard diploid chromosome number ($2n$) for the domestic horse (Equus caballus)?

(A) 60

(B) 62

(C) 64

(D) 78

2022

**43.** The river buffalo (Bubalus bubalis) differs in chromosome number from the swamp buffalo. What is the $2n$ of the river buffalo?
(A) 48
(B) 50
(C) 52
(D) 60

2023

**44.** G-banding of metaphase chromosomes primarily stains regions that are:
(A) GC-rich and early replicating
(B) Centromeric heterochromatin only
(C) AT-rich and late replicating
(D) Actively transcribed nucleolar organizers

2023

**45.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.
**Assertion (A):** Robertsonian translocations can reduce the diploid chromosome number of a population.
**Reason (R):** This translocation involves the fusion of two acrocentric chromosomes at their centromeres to form one metacentric chromosome.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2017

**46.** Which banding technique is specifically used to visualize constitutive heterochromatin, such as centromeres?
(A) G-banding
(B) Q-banding
(C) C-banding
(D) R-banding

2023

**47.** Avian karyotypes, such as that of the chicken ($2n = 78$), are uniquely characterized by the presence of:
(A) Only large metacentric chromosomes
(B) Macrochromosomes and microchromosomes
(C) Giant polytene salivary chromosomes
(D) Small circular bacterial-like chromosomes

2019

**48.** In livestock sex chromosome systems, birds differ from mammals because female birds are:
(A) XX (homogametic)
(B) XY (heterogametic)
(C) ZZ (homogametic)
(D) ZW (heterogametic)

2018

**49.** Minisatellites, often used in classical DNA fingerprinting, are technically referred to as:
(A) Variable Number Tandem Repeats (VNTRs)
(B) Short Tandem Repeats (STRs)
(C) Single Nucleotide Polymorphisms (SNPs)
(D) Long Terminal Repeats (LTRs)

2016

**50.** Microsatellites generally consist of repeating sequence motifs of what length?
(A) 1 to 6 base pairs
(B) 10 to 60 base pairs
(C) 100 to 500 base pairs
(D) Over 1000 base pairs

2001

**51.** Consider the following statements concerning microsatellites:
I. They exhibit high levels of allelic polymorphism.
II. They are codominant markers.
III. They undergo mutation primarily via slipped strand mispairing.
IV. They are widely distributed across the eukaryotic genome.
Which of the above statement(s) is/are correct?
(A) I, III and IV
(B) I, II and III
(C) II and IV
(D) I, II, III and IV

NODIA

2005

**52.** The predominant mutational mechanism responsible for variations in microsatellite allele length is:
(A) Unequal meiotic crossing over
(B) Autonomous transposon insertion
(C) Spontaneous cytosine deamination
(D) Slipped strand mispairing

2024

**53.** Minisatellite sequences are heavily clustered in which regions of human and livestock chromosomes?
(A) Centromeres
(B) Subtelomeric regions
(C) Exons of housekeeping genes
(D) Promoters

(C) Recombination error
(D) Slipped mismatch

2016
**121.** The $DGAT_1$ gene is a well-known major gene in dairy cattle associated primarily with:
(A) Meat tenderness
(B) Milk fat percentage and yield
(C) Horn development
(D) Coat color

2001
**122.** The causative mutation in the bovine $DGAT_1$ gene affecting milk traits is a non-synonymous substitution of:
(A) Arginine to Cysteine (R615C)
(B) Lysine to Alanine (K232A)
(C) Glutamic Acid to Valine (E6V)
(D) Glycine to Serine (G200S)

2013
**123.** The Myostatin (MSTN) gene negatively regulates muscle mass. Mutations in this gene cause the "double-muscling" phenotype in which cattle breed?
(A) Belgian Blue
(B) Holstein
(C) Jersey
(D) Brahman

2017
**124.** The molecular function of normal myostatin is to:
(A) Stimulate muscle cell proliferation
(B) Promote intramuscular fat deposition
(C) Inhibit myogenesis and muscle cell growth
(D) Synthesize glycogen

2018
**125.** The FecB (Booroola) major gene in sheep profoundly influences:
(A) Wool staple length
(B) Resistance to gastrointestinal nematodes
(C) Scrapie susceptibility
(D) Ovulation rate and litter size

2025
**126.** The FecB mutation is functionally located in the signaling pathway of which protein family?
(A) Growth Hormone Receptor (GHR)
(B) Insulin-like Growth Factor 1 (IGF-1)
(C) Bone Morphogenetic Proteins (BMPR1B)
(D) Myogenin (MYOG)

2008
**127.** The RYR1 gene mutation in pigs is economically important because it is the primary cause of:
(A) Porcine Reproductive and Respiratory Syndrome (PRRS)
(B) Porcine Stress Syndrome (PSS) and Pale Soft Exudative (PSE) meat
(C) Atrophic rhinitis
(D) African Swine Fever susceptibility

2023
**128.** The $RYR_1$ gene encodes the ryanodine receptor, which functions as a critical channel releasing which ion from the sarcoplasmic reticulum?
(A) Sodium
(B) Potassium
(C) Magnesium
(D) Calcium

2025
**129.** Callipyge (CLPG) is another muscle hypertrophy mutation in sheep. Its inheritance pattern is unique and known as:
(A) Polar overdominance (imprinting)
(B) X-linked recessive
(C) Simple Mendelian recessive
(D) Maternal mitochondrial

2010
**130.** The halothane test in pigs was historically used to phenotypically identify carriers of a mutation in which gene?
(A) MSTN
(B) $PRKAG_3$
(C) $RYR_1$
(D) $IGF_2$

2003
**131.** The $PRKAG_3$ gene mutation (Rendement Napole gene) in Hampshire pigs primarily affects:
(A) Muscle glycogen and meat quality
(B) Variation in total litter size
(C) Average carcass backfat thickness
(D) Phenotypic coat color variation

2022
**132.** The Weaver syndrome (Bovine Progressive Degenerative Myeloencephalopathy) in Brown Swiss cattle is an example of an autosomal recessive defect traced via:
(A) Quantitative flow cytometry
(B) Mitochondrial genome sequencing
(C) Fluorescent in situ hybridization
(D) Microsatellite linkage mapping

2010
**133.** The Major Histocompatibility Complex (MHC) in cattle is formally known as:
(A) SLA

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(C) Represent unique de novo mutations
(D) Inherit one allele from each parent

2019
**65.** When testing for parentage, the principle of exclusion relies on:
(A) Offspring allele not in either parent
(B) Sharing of common alleles in population
(C) Matching of maternal mitochondrial DNA
(D) High mutation rates creating new alleles

2015
**66.** What is the correct sequence of steps in classical RFLP-based DNA fingerprinting?
(A) Restriction digestion → Gel electrophoresis → Southern transfer → Probe hybridization
(B) Probe hybridization → Gel electrophoresis → Restriction digestion → Southern transfer
(C) Gel electrophoresis → Restriction digestion → Southern transfer → Probe hybridization
(D) Restriction digestion → Southern transfer → Gel electrophoresis → Probe hybridization

2003
**67.** The shift from classical VNTR fingerprinting to STR profiling was primarily driven by:
(A) Compatibility with PCR of degraded DNA
(B) Requirement of intact genomic DNA templates
(C) Insufficient levels of genetic polymorphism
(D) Specific patterns of maternal inheritance

2016
**68.** The "Power of Discrimination" in a forensic or livestock identification STR panel increases exponentially with:
(A) The length of the PCR primers
(B) The quantity of template DNA used
(C) The size of the gel electrophoresis tank
(D) The number of loci analyzed

2015
**69.** Multiplexing in DNA fingerprinting refers to:
(A) Sequencing the entire genome simultaneously
(B) Amplifying multiple STR loci in a single PCR reaction
(C) Using multiple restriction enzymes on one sample
(D) Running multiple samples in a single gel lane without distinct tags

2013
**70.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.
**Assertion (A):** STR-based parentage testing can definitively prove that a specific sire produced an offspring.
**Reason (R):** It is mathematically possible to exclude all other potential sires if enough highly polymorphic markers are used.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

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2008
**71.** The International Society for Animal Genetics (ISAG) recommends standard panels of which markers for cattle parentage testing?
(A) AFLPs
(B) RAPDs
(C) Minisatellites
(D) Microsatellites/SNPs

2012
**72.** In capillary electrophoresis for STR analysis, different alleles at the same locus are distinguished by:
(A) Migration time based on amplicon size
(B) Fluorescence emission wavelength
(C) Hybridization stringency
(D) Radioisotope halF-life

2005
**73.** Restriction Fragment Length Polymorphisms (RFLPs) map genetic variation caused primarily by:
(A) Slipped strand mispairing mechanisms
(B) SNPs at restriction endonuclease sites
(C) Expansion of trinucleotide repeat units
(D) Alternative splicing of mRNA transcripts

2005
**74.** Random Amplified Polymorphic DNA (RAPD) relies on the use of:
(A) Specific primers for known genes
(B) Short arbitrary primers (typically 10-mers)
(C) Oligo-dT primers
(D) Adapter-specific primers

2025
**75.** Consider the following statements regarding classical DNA markers:
I. RFLP is a codominant marker system.
II. RAPD requires prior knowledge of the target DNA sequence.
III. AFLP involves the ligation of specific adapter sequences.
Which of the above statement(s) is/are correct?

(A) Proteasome
(B) TAP transporter
(C) Invariant chain (Ii)
(D) Calnexin

2019
**147.** Endogenous viral proteins are degraded for MHC Class I presentation primarily by the:
(A) Proteasome
(B) Lysosome
(C) Golgi apparatus
(D) Endosome

2003
**148.** Peptides generated by the proteasome are transported into the endoplasmic reticulum for Class I binding via the:
(A) Sec61 translocon
(B) CLIP peptide
(C) HLA-DM molecule
(D) TAP1/TAP2 transporter

2003
**149.** Specific BoLA alleles have been historically linked with resistance or susceptibility to which cattle disease?
(A) Foot and Mouth Disease
(B) Bovine Leukemia Virus (Enzootic Bovine Leukosis)
(C) Rabies
(D) Bovine Spongiform Encephalopathy (BSE)

2009
**150.** In chickens, the $B_21$ haplotype of the B-complex confers strong genetic resistance against which viral disease?
(A) Newcastle Disease
(B) Avian Influenza
(C) Marek's Disease
(D) Infectious Bursal Disease

2007
**151.** The anchor residues of an antigenic peptide bind to specific pockets within the MHC:
(A) Cytoplasmic tail
(B) Transmembrane domain
(C) Beta-2 microglobulin interface
(D) Peptide-binding groove

2018
**152.** Cross-presentation allows dendritic cells to present exogenous antigens on:
(A) MHC Class I molecules
(B) MHC Class II molecules
(C) CD1 molecules
(D) Toll-like receptors

2025
**153.** A critical function of MHC molecules in maintaining selF-tolerance occurs during T cell maturation in the:
(A) Bone marrow
(B) Thymus
(C) Spleen
(D) Lymph nodes

2024
**154.** Certain SLA haplotypes in pigs have been associated with immune responses to which economically devastating virus?
(A) Classical Swine Fever Virus
(B) Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)
(C) Porcine Epidemic Diarrhea Virus
(D) Swine Influenza Virus

2021
**155.** Natural Killer (NK) cells identify and destroy infected host cells primarily when those host cells:
(A) Downregulate MHC Class I expression
(B) Overexpress MHC Class II
(C) Secrete high levels of antibodies
(D) Fail to undergo apoptosis

2017
**156.** The phenomenon where an MHC molecule can bind many different peptides, but a specific peptide only binds certain MHC alleles, highlights the MHC's:
(A) Monomorphic nature
(B) Enzymatic activity
(C) Broad specificity
(D) Intracellular location

2018
**157.** The mitochondrial genome (mtDNA) of farm animals is structurally organized as a:
(A) Linear double-stranded DNA
(B) Circular single-stranded DNA
(C) Linear single-stranded RNA
(D) Circular double-stranded DNA

2019
**158.** The standard size of the complete mitochondrial genome in most livestock species is approximately:
(A) 1.6 kb
(B) 16.5 kb
(C) 160 kb
(D) 1.6 Mb

2004
**159.** Mitochondrial DNA follows a strict pattern of:
(A) Paternal inheritance

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2002

**87.** Consider the following statements concerning SNPs:

I. They are predominantly codominant markers.
II. They have higher mutation rates than microsatellites.
III. They are highly amenable to automated high-throughput genotyping.
IV. Synonymous SNPs alter the amino acid sequence of a protein.

Which of the above statement(s) is/are correct?

(A) I and II
(B) I and III
(C) II
(D) IV

2017

**88.** A single nucleotide polymorphism resulting from the substitution of an Adenine (A) for a Guanine (G) is termed a:

(A) Transition
(B) Transversion
(C) Frameshift
(D) Deletion

2004

**89.** Arrange the following steps of high-density SNP array genotyping in the correct order:

I. Allele-specific single base extension
II. Whole-genome DNA amplification
III. Enzymatic fragmentation and precipitation
IV. Hybridization to oligonucleotide probes on the bead chip

(A) II, III, IV, I
(B) III, II, I, IV
(C) II, IV, III, I
(D) I, II, III, IV

2010

**90.** High-density SNP genotyping arrays fundamentally rely on the principle of:

(A) Capillary electrophoresis sizing
(B) Sequence-specific nucleic acid hybridization
(C) Reverse transcription of RNA
(D) De novo whole-genome assembly

2009

**91.** Illumina BeadChip arrays distinguish between SNP alleles predominantly using:

(A) Primer extension using fluorescent ddNTPs
(B) Targeted restriction enzyme cleavage events
(C) Matrix-assisted laser desorption/ionization
(D) Recognition of slipped strand mismatch sites

2015

**92.** A SNP involving the replacement of a Cytosine (C) with an Adenine (A) is chemically classified as a:

(A) Transition
(B) Translocation
(C) Transversion
(D) Transposition

2023

**93.** The following question consists of two statements — Assertion (A) and Reason (R). Answer the question by selecting the appropriate option.

**Assertion (A):** SNPs have largely replaced microsatellites in genomic selection programs for livestock.

**Reason (R):** SNPs are biallelic markers that are easy to automate and occur at high density across the genome.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2023

**94.** A locus is generally considered a valid SNP for population genetics if the Minor Allele Frequency (MAF) is greater than:

(A) 0.01%
(B) 1%
(C) 20%
(D) 50%

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2001

**95.** A SNP occurring within a coding region that changes the encoded amino acid is defined as:

(A) Synonymous
(B) Non-synonymous
(C) Intergenic
(D) Intronic

2004

**96.** Genome-wide association studies (GWAS) heavily utilize high-density SNP arrays to identify markers in:

(A) Complete independence from surrounding genes
(B) Mitochondrial D-loops only
(C) Conserved non-polymorphic zones
(D) Linkage disequilibrium with causative quantitative trait loci (QTL)

2009
**97.** In genetic linkage mapping, distances are measured using which standard unit?
(A) Centimorgans (cM)
(B) Base pairs (bp)
(C) Nanometers (nm)
(D) Kilodaltons (kDa)

2021
**98.** One Centimorgan (cM) represents a recombination frequency between two linked loci of approximately:
(A) 0.1%
(B) 1%
(C) 10%
(D) 50%

2003
**99.** Consider the following statements regarding genetic mapping:
I. Physical maps measure distance in base pairs.
II. Linkage analysis utilizes meiotic recombination events.
III. A LOD score is a statistical test for linkage.
IV. Double crossovers lead to underestimation of genetic distance if not corrected.
Which of the above statement(s) is/are correct?
(A) I and II
(B) II, III and IV
(C) I, II, III and IV
(D) II and III

2002
**100.** Physical genome maps, as opposed to genetic linkage maps, measure distances in:
(A) Centimorgans
(B) Recombination units
(C) LOD scores
(D) Base pairs (bp)

2015
**101.** Match the items in List I with the items in List II:
List I: (Mapping Term)
P. Recombination frequency
Q. LOD Score
R. Physical map
S. Genetic map
List II: (Definition)
1. Base pair distance between loci
2. Based on meiotic crossover rates
3. Statistical measure of linkage likelihood
4. Proportion of non-parental gametes
(A) P-IV, Q-III, R-I, S-II
(B) P-II, Q-I, R-III, S-IV
(C) P-III, Q-IV, R-II, S-I
(D) P-I, Q-II, R-IV, S-III

2002
**102.** The LOD score formula calculates the logarithm to the base 10 of the odds ratio comparing the probability of:
(A) Mutation versus wild-type alleles
(B) Dominance versus epistasis
(C) Linkage at a specific recombination fraction versus independent assortment
(D) Physical distance versus genetic distance

2018
**103.** A calculated LOD score of 3.0 establishes that the likelihood of linkage is:
(A) 3 times greater than independent assortment
(B) 30 times greater than independent assortment
(C) 1,000 times greater than independent assortment
(D) 10,000 times greater than independent assortment

2004
**104.** In a mapping population, two loci segregating with a recombination frequency of 50% are considered to be:
(A) Tightly linked
(B) Separated by exactly 5 cM
(C) Genetically unlinked
(D) Always on different chromosomes

2015
**105.** Haldane's mapping function is applied to recombination frequencies to correct for:
(A) Point mutations occurring during meiosis
(B) Multiple and unobserved crossover events
(C) Errors in automated genotyping
(D) Epigenetic modifications

2008
**106.** Which mapping population structure is most extensively utilized in dairy cattle linkage studies?
(A) Recombinant inbred lines
(B) HalF-sib family designs
(C) Progeny of inbred lines
(D) Doubled haploid groups

2022
**107.** Kosambi's mapping function differs from Haldane's primarily by incorporating:
(A) Crossover interference
(B) The physical mass of the chromosome
(C) Mutation rates of SNPs
(D) Base pair length

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2025
**108.** Radiation hybrid mapping bridges genetic and physical maps by utilizing:
(A) Meiotic recombination in target families
(B) Whole-genome shotgun sequencing
(C) X-ray induced chromosomal breaks and somatic cell fusion
(D) Restriction enzyme partial digests

2001
**109.** Marker-Assisted Selection (MAS) is fundamentally based on identifying DNA markers that are:
(A) Phenotypically expressed as physical traits
(B) Located strictly within mitochondrial DNA
(C) Always directly causing the trait
(D) In strong linkage disequilibrium with Quantitative Trait Loci (QTL)

2002
**110.** MAS is generally most cost-effective and advantageous when applied to phenotypic traits exhibiting:
(A) High heritability and ease of measurement
(B) Purely monogenic Mendelian inheritance
(C) Low heritability, sex-limitation, or requiring late-life measurement
(D) Zero phenotypic variation in the population

2024
**111.** A Quantitative Trait Locus (QTL) is defined as a:
(A) Single base pair mutation causing a lethal disease
(B) Genomic region strongly associated with variation in a continuously distributed trait
(C) Non-coding RNA regulating translation
(D) Region devoid of any genetic markers

2015
**112.** The precision of QTL mapping in livestock is primarily limited by:
(A) The number of chromosomes
(B) The amount of highly repetitive DNA
(C) Mitochondrial inheritance patterns
(D) The density of markers and the size of the mapping population

2007
**113.** In MAS, the "marker effect" quantifies:
(A) The proportion of phenotypic variance explained by the marker genotype
(B) The physical distance between two markers
(C) The LOD score for linkage
(D) The mutation rate of the marker

2018
**114.** Introgression of a desirable QTL into a recipient breeding line using MAS is technically referred to as:
(A) Genomic selection
(B) Transgenesis
(C) Marker-assisted backcrossing (MABC)
(D) Mutagenesis

2017
**115.** A significant limitation of early marker-assisted selection programs was:
(A) Variable linkage phase across families
(B) High frequency of lethal marker alleles
(C) Incompatibility of markers with PCR steps
(D) Restriction of genotyping to female animals

2009
**116.** Fine mapping of a broad QTL region aims to narrow the interval to identify the:
(A) Centromere location
(B) Telomeric repeats
(C) Pseudoautosomal region
(D) Quantitative Trait Nucleotide (QTN) or causal mutation

2011
**117.** Interval mapping for QTL detection improves upon single-marker analysis by utilizing:
(A) Only unlinked markers
(B) The estimated recombination frequencies between adjacent flanking markers
(C) Pedigree data exclusively without genotypes
(D) Transcriptomic data directly

2007
**118.** Which concept superseded MAS by estimating breeding values using dense, genome-wide marker sets simultaneously?
(A) Phenotypic selection
(B) Classical progeny testing
(C) Genomic Selection (GS)
(D) FISH mapping

NODIA

2015
**119.** In QTL analysis, a genome-wide significance threshold is applied to correct for:
(A) Low marker density
(B) Errors in phenotypic recording
(C) Incomplete penetrance
(D) Multiple testing of thousands of markers

2007
**120.** A false positive detection of a QTL is statistically referred to as a:
(A) Type I error
(B) Type II error

(A) I and II
(B) I and III
(C) I
(D) III

2014
**76.** The major technical limitation of RAPD markers in genetic analysis is:
(A) Requirement of radioactive isotopes
(B) High cost of primer synthesis
(C) Low reproducibility between laboratories
(D) Codominant mode of inheritance

2021
**77.** Amplified Fragment Length Polymorphism (AFLP) successfully integrates the principles of:
(A) Northern blotting and PCR
(B) Microarrays and RAPD
(C) Sanger sequencing and STRs
(D) RFLP and PCR

2010
**78.** Which of the following marker systems typically demonstrates dominant inheritance, preventing the differentiation of heterozygotes from dominant homozygotes?
(A) RFLP
(B) Microsatellite
(C) RAPD
(D) SNP

2024
**79.** Match the items in List I with the items in List II:
List I: (DNA Marker)
P. RFLP
Q. RAPD
R. AFLP
S. SNP
List II: (Defining Feature)
1. Uses arbitrary 10-mer primers
2. Requires restriction digestion and adapters
3. Single nucleotide variation
4. Requires Southern blotting in classical form
(A) P-IV, Q-I, R-II, S-III
(B) P-I, Q-IV, R-III, S-II
(C) P-IV, Q-II, R-I, S-III
(D) P-II, Q-III, R-IV, S-I

2018
**80.** The standard AFLP protocol utilizes a combination of two restriction enzymes, typically:
(A) Two frequent cutters (e.g., TaqI and MseI)
(B) Two rare cutters (e.g., NotI and SfiI)
(C) A blunt-end cutter and a nicking endonuclease
(D) One rare cutter (e.g., EcoRI) and one frequent cutter (e.g., MseI)

2001
**81.** In AFLP analysis, the selective amplification step achieves specificity by adding:
(A) Arbitrary nucleotides at the 3' primer end
(B) Fluorophores attached to the 5' primer end
(C) Poly-A tail synthesis on restriction fragments
(D) Uracil base incorporation instead of thymine

2015
**82.** Which of the following markers requires the largest amount of high-quality, high-molecular-weight genomic DNA?
(A) RAPD
(B) Microsatellite PCR
(C) Classical RFLP
(D) SNP arrays

2017
**83.** The main advantage of AFLP over RAPD and classical RFLP is its capacity to:
(A) High band polymorphism without sequencing
(B) Direct determination of precise allele sequences
(C) Consistent differentiation of codominant alleles
(D) Elimination of the need for restriction enzymes

2022
**84.** Which marker system utilizes polymorphism arising from the presence or absence of a restriction site, combined with variation in the length of the intervening sequence?
(A) RAPD
(B) ISSR
(C) SCAR
(D) RFLP

2004
**85.** Single Nucleotide Polymorphisms (SNPs) are the most abundant genetic variation, typically occurring in mammalian genomes once every:
(A) 10 to 50 bp
(B) 500 to 1,000 bp
(C) 10,000 to 50,000 bp
(D) 100,000 bp

2012
**86.** The vast majority of SNPs found in livestock populations are:
(A) Diallelic (biallelic)
(B) Triallelic
(C) Tetra-allelic
(D) Hypervariable with many length alleles

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(B) BoLA
(C) B-complex
(D) OLA

2023
**134.** Swine Leukocyte Antigen (SLA) is the specific designation for the MHC system in:
(A) Sheep
(B) Goats
(C) Pigs
(D) Horses

2019
**135.** The avian MHC, notably studied in chickens, is known as the:
(A) BoLA
(B) ELA
(C) DLA
(D) B-complex

2011
**136.** MHC Class I molecules primarily present endogenous antigens to which class of immune cells?
(A) CD8+ Cytotoxic T cells
(B) CD4+ T helper cells
(C) B lymphocytes
(D) Macrophages

2011
**137.** Which protein structurally associates with the heavy chain of MHC Class I but is not encoded within the MHC genomic region?
(A) Tapasin
(B) Beta-2 microglobulin
(C) Invariant chain
(D) CD4

2007
**138.** MHC Class II molecules are characteristically expressed exclusively on:
(A) All nucleated cells
(B) Professional antigen-presenting cells (e.g., dendritic cells, macrophages)
(C) Erythrocytes
(D) Platelets

2007
**139.** The most striking evolutionary characteristic of the MHC region across mammalian species is its extreme:
(A) Polymorphism at peptide-binding clefts
(B) Extreme conservation and lack of variation
(C) High susceptibility to retroviral integration
(D) Repetitive composition lacking coding genes

2017
**140.** In the chicken B-complex, the structural arrangement differs from mammals primarily because it is:
(A) Larger size with numerous pseudogenes
(B) Total absence of MHC Class I genes
(C) Highly compact with no Class III region
(D) Location on the mitochondrial genome

2010
**141.** The peptide-binding cleft of an MHC Class II molecule is formed by:
(A) Only the alpha-1 domain
(B) The alpha-1 and alpha-2 domains of a single heavy chain
(C) Beta-2 microglobulin alone
(D) The alpha-1 and beta-1 domains of two distinct chains

2017
**142.** The Class III region of the MHC predominantly contains genes encoding components for the:
(A) T-cell receptor
(B) Immunoglobulin heavy chains
(C) Complement system and inflammatory cytokines
(D) Perforins and granzymes

2005
**143.** Which technique is most commonly employed for high-resolution SLA or BoLA typing today?
(A) Serological microcytotoxicity assays
(B) PCR-sequence based typing (SBT)
(C) Southern blotting
(D) Protein electrophoresis

2009
**144.** The high level of polymorphism in MHC genes is maintained evolutionarily by:
(A) Purifying selection
(B) Genetic drift alone
(C) Founder effects
(D) Heterozygote advantage and frequency-dependent selection

2006
**145.** MHC Class II molecules present exogenous antigens derived from extracellular pathogens to:
(A) CD4+ T helper cells
(B) CD8+ T cells
(C) Natural Killer (NK) cells
(D) Neutrophils

2010
**146.** Inside the antigen-presenting cell, MHC Class II molecules are prevented from binding premature peptides in the ER by the:

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2016

**54.** Microsatellites are highly favored for parentage testing in livestock because they are:
(A) Monomorphic and highly stable
(B) Dominant and easily visualized without PCR
(C) Codominant and highly polymorphic
(D) Maternally inherited

2003

**55.** Match the items in List I with the items in List II:
List I: (Repeat Marker)
P. Minisatellite
Q. Microsatellite
R. Dinucleotide repeat
S. Telomeric repeat
List II: (Key Attribute)
1. Slipped strand mispairing
2. VNTR
3. TTAGGG array
4. Most common STR motif in mammals
(A) P-II, Q-I, R-IV, S-III
(B) P-I, Q-II, R-III, S-IV
(C) P-II, Q-III, R-I, S-IV
(D) P-III, Q-I, R-IV, S-II

2006

**56.** Unequal crossing over during meiosis is the primary mutational driver for which class of tandem repeats?
(A) Minisatellites
(B) Microsatellites
(C) Mononucleotide repeats
(D) SNPs

2013

**57.** Which repeat motif constitutes the most abundant class of microsatellites in livestock genomes?
(A) Mononucleotide
(B) Dinucleotide
(C) Tetranucleotide
(D) Hexanucleotide

2016

**58.** PCR-amplified microsatellite alleles are typically resolved and sized using:
(A) Southern blotting
(B) Capillary electrophoresis
(C) Density gradient centrifugation
(D) Microarray hybridization

2004

**59.** The inheritance pattern of microsatellite markers allows differentiation of heterozygotes from homozygotes, making them:
(A) Codominant markers
(B) Epistatic markers
(C) Dominant markers
(D) Recessive markers

2023

**60.** Microsatellite instability (MSI) is primarily caused by defects in which cellular system?
(A) Non-homologous end joining
(B) Base excision repair
(C) Mismatch repair
(D) Nucleotide excision repair

2006

**61.** The concept and methodology of DNA fingerprinting were pioneered in 1984 by:
(A) Kary Mullis
(B) Frederick Sanger
(C) James Watson
(D) Alec Jeffreys

2012

**62.** Original DNA fingerprinting utilized radioactive probes designed to hybridize to:
(A) Single-copy structural genes
(B) Specific SNP loci
(C) Core sequences of minisatellites
(D) Mitochondrial D-loop regions

NODIA

2012

**63.** Consider the following statements regarding modern DNA fingerprinting in livestock:
I. It relies heavily on multiplex PCR of STRs.
II. It requires small quantities of DNA compared to RFLP.
III. It generates codominant genotype data.
IV. Allele sizes are precisely measured using fluorescent tags.
V. It can resolve paternity disputes with $> 99\%$ probability.
Which of the above statement(s) is/are correct?
(A) I, II, III and V
(B) I, III, IV and V
(C) I, II, III, IV and V
(D) II, III and V

2018

**64.** In livestock parentage testing using microsatellites, an offspring's alleles at a given locus must:
(A) Match the sire alleles exclusively
(B) Match the dam alleles exclusively

(B) Maternal inheritance
(C) Mendelian biparental inheritance
(D) X-linked recessive inheritance

2006
**160.** How many protein-coding genes are universally encoded by the mammalian mitochondrial genome?
(A) 13
(B) 22
(C) 37
(D) over 100

2010
**161.** The 13 proteins encoded by mtDNA are exclusively subunits of complexes involved in:
(A) Oxidative phosphorylation
(B) DNA replication
(C) Glycolysis
(D) The citric acid cycle

2018
**162.** Alongside protein-coding genes, the mammalian mtDNA encodes how many transfer RNAs (tRNAs)?
(A) 2
(B) 13
(C) 22
(D) 64

2023
**163.** The mutation rate of mitochondrial DNA compared to nuclear DNA is generally:
(A) 10 to 100 times slower
(B) Exactly the same
(C) 10 to 17 times higher
(D) Zero, as mtDNA does not mutate

2023
**164.** The higher mutation rate of mtDNA is partially attributed to the:
(A) Total absence of DNA polymerases
(B) Lack of a protective nuclear envelope
(C) Total absence of non-coding introns
(D) Lack of histones and efficient repair

2025
**165.** The presence of more than one distinct type of mitochondrial DNA within a single cell or individual is termed:
(A) Homoplasmy
(B) Heteroplasmy
(C) Polyploidy
(D) Aneuploidy

2023
**166.** A unique feature of the mammalian mitochondrial genetic code is that UGA encodes:
(A) Stop
(B) Methionine
(C) Tryptophan
(D) Arginine

2008
**167.** The two strands of the circular mitochondrial genome are traditionally designated as the:
(A) Leading and Lagging strands
(B) Sense and Antisense strands
(C) Watson and Crick strands
(D) Heavy (H) and Light (L) strands

2006
**168.** Mitochondrial genes entirely lack which structural feature typical of nuclear eukaryotic genes?
(A) Introns
(B) Exons
(C) Start codons
(D) Promoters

2025
**169.** The most highly polymorphic, non-coding region of the mitochondrial genome used for phylogenetic analysis is the:
(A) Cytochrome b gene
(B) Displacement loop (D-loop)
(C) 16S rRNA gene
(D) ATP synthase 6 gene

2024
**170.** The mtDNA D-loop is scientifically critical because it contains the:
(A) Primary translation start sites
(B) Origin of replication for the Heavy strand and promoters for transcription
(C) Genes for the 22 tRNAs
(D) Introns necessary for splicing

2018
**171.** Sequencing the D-loop allows researchers to primarily investigate a livestock species':
(A) Maternal lineage and domestication history
(B) Muscle development potential
(C) Resistance to viral infections
(D) Coat color inheritance

2012
**172.** Based on mtDNA D-loop sequence analysis, it is established that Bos taurus and Bos indicus cattle:
(A) Belong to the same maternal mitochondrial lineage

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(C) tRNA
(D) rRNA

2001
**268.** For Streptococcus pyogenes Cas9 (SpCas9) to successfully cleave DNA, the target sequence must be immediately followed at its 3' end by a short sequence called the:
(A) Repeat-Variable Diresidue (RVD)
(B) Zinc Finger Motif
(C) Protospacer Adjacent Motif (PAM)
(D) Poly-A tail

2006
**269.** The canonical PAM sequence required by wild-type SpCas9 is:
(A) 5'-TTTV-3'
(B) 5'-NGG-3'
(C) 5'-NNNNGATT-3'
(D) 5'-CCG-3'

2024
**270.** Cas9 acts as a dual-RNA-guided DNA endonuclease. It introduces a double-strand break using two distinct nuclease domains named:
(A) FokI and EcoRI
(B) Cas1 and Cas2
(C) RuvC and HNH
(D) $RAG_1$ and $RAG_2$

2006
**271.** The DNA cleavage generated by wild-type SpCas9 typically results in:
(A) 5' staggered overhangs
(B) Blunt ends approximately 3 bp upstream of the PAM
(C) 3' staggered overhangs
(D) Single-stranded nicks only

2002
**272.** A catalytically "dead" Cas9 (dCas9) can be created by mutating the nuclease domains. dCas9 is primarily used for:
(A) More efficient DNA cutting
(B) Homology-directed repair enhancement
(C) Destroying RNA transcripts
(D) Targeted gene silencing (CRISPRi) or activation (CRISPRa) without cutting DNA

2025
**273.** The target-specific "spacer" sequence within the sgRNA is typically how many nucleotides long?
(A) 10 nt
(B) 20 nt
(C) 50 nt
(D) 100 nt

2005
**274.** Base editors developed from CRISPR systems fuse a catalytically impaired Cas nuclease to a:
(A) Reverse transcriptase
(B) DNA polymerase
(C) Deaminase enzyme
(D) Methyltransferase

2004
**275.** Prime Editing, a newer highly precise CRISPR derivative, allows targeted insertions, deletions, and all 12 types of base substitutions by combining Cas9 with a:
(A) Reverse transcriptase and a pegRNA
(B) FokI nuclease
(C) Cytosine deaminase
(D) Transposase

2013
**276.** Multiplex genome editing (targeting multiple genes simultaneously) is vastly easier with CRISPR than TALENs or ZFNs because it only requires:
(A) Multiple distinct Cas9 proteins
(B) Fusing multiple FokI domains together
(C) Injecting different metal ions
(D) Delivering multiple different sgRNAs with a single Cas9 protein

2025
**277.** Porcine Reproductive and Respiratory Syndrome (PRRS) causes massive economic losses worldwide. The PRRS virus primary entry receptor on porcine alveolar macrophages is:
(A) CD4
(B) CD163
(C) $ACE_2$
(D) CCR5

2001
**278.** To create PRRS-resistant pigs, CRISPR-Cas9 was used to specifically knock out or modify which domain of the CD163 protein?
(A) Transmembrane domain
(B) Cytoplasmic tail
(C) Scavenger receptor cysteine-rich (SRCR) domain 5
(D) Signal peptide

2012
**279.** The advantage of specifically deleting SRCR domain 5 of CD163 rather than the entire gene is that the pigs:
(A) Gain increased muscle mass
(B) Become resistant to all known viruses
(C) Produce more milk

NODIA

is required, which method replaces poly-A selection?
(A) Ribosomal RNA depletion (Ribo-Zero)
(B) Poly-C tagging
(C) Size exclusion chromatography
(D) Exonuclease digestion

2012

**185.** During RNA-Seq library preparation, isolated RNA must first be converted into a more stable form. This step is known as:
(A) In vitro transcription
(B) Reverse transcription into complementary DNA (cDNA)
(C) RNA ligation
(D) Bisulfite conversion

2005

**186.** The process of attaching short, synthesized DNA fragments to the ends of cDNA for sequencing platform compatibility is called:
(A) Tailing
(B) Adapter ligation
(C) Amplification
(D) Fragmentation

2005

**187.** Strand-specific RNA-Seq libraries are highly advantageous because they allow researchers to:
(A) Distinguish between overlapping transcripts from the sense and antisense strands
(B) Avoid the use of reverse transcriptase
(C) Sequence the poly-A tail infinitely
(D) Directly sequence RNA molecules without making cDNA

2024

**188.** Which next-generation sequencing technology is predominantly used for short-read RNA-Seq in livestock research?
(A) Sanger Sequencing
(B) Maxam-Gilbert Sequencing
(C) Illumina Sequencing-by-Synthesis
(D) Oxford Nanopore Direct RNA

2016

**189.** Single-cell RNA-Seq (scRNA-Seq) differs from bulk RNA-Seq by fundamentally providing insights into:
(A) Average gene expression across a whole tissue
(B) Only ribosomal RNA populations
(C) Complete genomic DNA sequences of a single cell
(D) Transcriptional profiles and heterogeneity of individual cells

2006

**190.** Multiplexing multiple RNA-Seq samples onto a single sequencing lane is achieved by:
(A) Mixing RNA before extraction
(B) Running them on separate machines
(C) Adding unique index barcodes to the adapters of each sample
(D) Ignoring strand specificity

2022

**191.** Fragmentation of RNA or cDNA during library preparation is essential because short-read sequencing platforms optimally read fragments of approximately:
(A) 20-50 bp
(B) 150-300 bp
(C) 1,000-2,000 bp
(D) 10,000 bp

2008

**192.** Assessing RNA integrity before library prep involves generating an RIN score using an Agilent Bioanalyzer. A high-quality RNA sample for RNA-Seq typically has an RIN score of:
(A) $> 8$
(B) $\sim 4$
(C) $< 2$

2002

**193.** The first bioinformatics step after obtaining raw RNA-Seq data from a sequencer is typically:
(A) Differential expression analysis
(B) Read alignment to a reference genome
(C) Quality control (QC) and adapter trimming
(D) Pathway enrichment analysis

2012

**194.** A widely used software tool for conducting fast quality control checks on raw FASTQ files is:
(A) DESeq2
(B) STAR
(C) FastQC
(D) edgeR

2007

**195.** Unlike DNA alignment, aligning RNA-Seq reads to a eukaryotic reference genome requires a specialized "splice-aware" aligner. This is to account for:
(A) Spliced out introns
(B) The poly-A tail
(C) Exon duplication
(D) Chromosomal translocations

2010

**196.** Which of the following is a prominent splice-aware read aligner used in RNA-Seq pipelines?

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2001

**220.** Methanogenesis in the rumen, which produces the greenhouse gas methane, is exclusively carried out by microorganisms belonging to the domain:
(A) Bacteria
(B) Eukarya
(C) Archaea
(D) Fungi

2010

**221.** Rumen methanogens produce methane primarily by reducing carbon dioxide ($CO_2$) using:
(A) Oxygen ($O_2$)
(B) Nitrogen gas ($N_2$)
(C) Sulfide ($H_2S$)
(D) Hydrogen gas ($H_2$)

2024

**222.** The synthesis of methane in the rumen represents a significant physiological process because it:
(A) Provides the host with dietary glucose
(B) Constitutes a loss of 2% to 12% of the animal's gross energy intake
(C) Regulates rumen temperature
(D) Prevents bloat

2012

**223.** Applying metagenomics to the rumen microbiome has identified strong correlations between specific microbial community profiles and the host animal's:
(A) Coat color
(B) Feed Conversion Ratio (FCR) / Residual Feed Intake (RFI)
(C) Horn length
(D) Blood type

2007

**224.** Which compound, often studied via metagenomic impact on the rumen, acts as a potent specific inhibitor of the methanogenic enzyme methyl-coenzyme M reductase?
(A) Monensin
(B) Bromoform
(C) Ionophore antibiotics
(D) 3-Nitrooxypropanol (3-NOP)

2011

**225.** Seaweed supplementation (e.g., Asparagopsis taxiformis) reduces enteric methane emissions primarily due to the presence of:
(A) Bromoform
(B) Tannins
(C) Saponins
(D) Essential oils

2003

**226.** The shift from a forage-based to a high-grain concentrate diet dramatically changes the rumen microbiome, significantly increasing the risk of:
(A) Rumen alkalosis
(B) Hypocalcemia
(C) Subacute Ruminal Acidosis (SARA)
(D) Ketosis

2007

**227.** Increased production of which VFA in the rumen acts as a hydrogen sink, inversely correlating with methane production?
(A) Propionate
(B) Acetate
(C) Butyrate
(D) Valerate

2019

**228.** WGS metagenomics of the rumen has successfully identified novel enzymes crucial for breaking down recalcitrant plant cell walls. These enzymes are collectively called:
(A) Restriction endonucleases
(B) Peptidases
(C) Polymerases
(D) Carbohydrate-Active enZymes (CAZymes)

2010

**229.** All modern programmable genome editing technologies fundamentally rely on inducing a precise:
(A) Single-strand DNA nick
(B) Double-Strand Break (DSB)
(C) Cytosine methylation
(D) Thymine dimer

2017

**230.** Following a targeted DSB, the cell's predominant, error-prone repair pathway that often leads to gene knockout is:
(A) Homology-Directed Repair (HDR)
(B) Base Excision Repair (BER)
(C) Non-Homologous End Joining (NHEJ)
(D) Mismatch Repair (MMR)

2001

**231.** NHEJ typically introduces small mutations at the DSB site collectively known as:
(A) SNPs
(B) Translocations
(C) Inversions
(D) Indels (insertions/deletions)

NODIA

2004
**208.** In 16S metagenomics, similar sequences are classically clustered into units representing approximate species level groupings called:
(A) Sequence Read Archives (SRAs)
(B) Single Nucleotide Variants (SNVs)
(C) Open Reading Frames (ORFs)
(D) Operational Taxonomic Units (OTUs)

2001
**209.** Modern 16S analysis often utilizes Amplicon Sequence Variants (ASVs) instead of OTUs to provide:
(A) Exact sequence-level resolution distinguishing single nucleotide differences
(B) A lower resolution overview of phyla
(C) Whole-genome assemblies
(D) RNA expression data

2016
**210.** Whole-Genome Shotgun (WGS) metagenomics differs from 16S sequencing by:
(A) Amplifying only the 18S rRNA gene
(B) Being significantly cheaper and requiring less computing power
(C) Sequencing all DNA in the sample, capturing functional genes and non-bacterial taxa
(D) Generating strictly circular genomes

2008
**211.** Which metric describes the number of different microbial species present within a single sample (local diversity)?
(A) Alpha diversity
(B) Beta diversity
(C) Gamma diversity
(D) Zeta diversity

2009
**212.** Beta diversity in metagenomic studies is used to measure:
(A) The total number of sequences generated
(B) The mutation rate of the 16S gene
(C) The total biomass of the sample
(D) The difference in microbial community composition between different samples or environments

2018
**213.** A major advantage of WGS metagenomics over 16S amplicon sequencing is the ability to directly profile:
(A) Only bacterial phylogeny
(B) Antibiotic resistance genes and metabolic pathways
(C) Host animal pedigree
(D) The age of the microorganisms

2012
**214.** The assembly of individual microbial genomes from complex WGS metagenomic data results in:
(A) Plasmid maps
(B) Karyotypes
(C) Metagenome-Assembled Genomes (MAGs)
(D) Amplicon Sequence Variants

2010
**215.** Uncultured, uncharacterized microbes identified solely via metagenomic sequencing are often referred to as microbial:
(A) “Weeds”
(B) “Satellites”
(C) “Orphans”
(D) “Dark matter”

2001
**216.** The rarefaction curve in a metagenomics study plots sequencing depth against the number of observed species to assess:
(A) Whether the microbial diversity in the sample has been exhaustively sequenced
(B) PCR duplication rates
(C) The length of the reads
(D) Contamination levels

2009
**217.** The ruminant stomach chamber primarily responsible for massive microbial fermentation of plant polysaccharides is the:
(A) Abomasum
(B) Rumen
(C) Omasum
(D) Reticulum

2003
**218.** The dominant bacterial phyla consistently found in the rumen microbiome of cattle and sheep are:
(A) Proteobacteria and Actinobacteria
(B) Bacteroidetes and Firmicutes
(C) Cyanobacteria and Spirochaetes
(D) Fusobacteria and Tenericutes

2011
**219.** Rumen microbes ferment complex carbohydrates to produce energy substrates for the host animal. These substrates are predominantly:
(A) Volatile Fatty Acids (VFAs) like acetate, propionate, and butyrate
(B) Long-chain fatty acids
(C) Simple sugars like glucose and fructose
(D) Essential amino acids exclusively

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(A) BWA-MEM
(B) Bowtie2
(C) BLASTp
(D) STAR

2018
**197.** After alignment, the pipeline requires quantifying how many reads map to each gene. A common tool for read counting is:
(A) FastQC
(B) FeatureCounts
(C) Trimmomatic
(D) ClustalW

2022
**198.** Raw read counts must be normalized to compare gene expression between samples accurately. Normalization typically adjusts for:
(A) Intron length and GC content
(B) RNA polymerase speed
(C) Sequencing depth and gene length
(D) Transposon density

2011
**199.** FPKM (Fragments Per Kilobase of transcript per Million mapped reads) and TPM (Transcripts Per Million) are both:
(A) Raw count metrics
(B) Quality score metrics (Phred scores)
(C) Alignment mapping quality scores
(D) Normalized gene expression metrics

2021
**200.** The statistical analysis of read counts to identify significantly up- or down-regulated genes between two conditions is called:
(A) Differential Gene Expression (DGE) analysis
(B) De novo assembly
(C) Variant calling
(D) Homology modeling

2004
**201.** DESeq2 and edgeR, two popular R packages for DGE analysis, model RNA-Seq count data using which statistical distribution?
(A) Normal distribution
(B) Negative Binomial distribution
(C) Poisson distribution
(D) Uniform distribution

2022
**202.** A Volcano Plot is frequently used in RNA-Seq to visualize:
(A) Sequence read length vs quality
(B) $LoG_2$ fold-change versus statistical significance (p-value)
(C) Number of exons per gene
(D) GC bias across the chromosome

2022
**203.** Gene Ontology (GO) and KEGG pathway enrichment analyses are performed post-DGE to:
(A) Identify overrepresented biological pathways
(B) Identify the physical location of the genes
(C) Design oligonucleotide primers for qPCR
(D) Calculate the global read mapping percentage

2014
**204.** "Transcriptome Assembly" (e.g., via Trinity) is performed when:
(A) Identifying SNPs in the promoter
(B) Mapping raw reads to known genes
(C) A high-quality reference genome is unavailable
(D) Visualizing read coverage in a browser

2006
**205.** Metagenomics fundamentally differs from traditional genomics because it involves the sequencing of genomic DNA from:
(A) A single isolated and cultured bacterial colony
(B) The nuclear DNA of a host animal
(C) Only the viral particles in a sample
(D) An entire community of microorganisms extracted directly from an environment

2005
**206.** 16S rRNA amplicon sequencing is utilized primarily to determine:
(A) The complete metabolic capabilities of a microbiome
(B) The presence of specific fungal pathogens
(C) The taxonomic composition and relative abundance of bacterial and archaeal populations
(D) The viral load in a sample

2001
**207.** The 16S rRNA gene is ideal for microbial phylogeny because it contains both:
(A) Highly conserved regions for universal primer binding and hypervariable regions for species identification
(B) Promoters and terminators
(C) Virulence factors and antibiotic resistance genes
(D) Introns and exons

NODIA

2019
**232.** To introduce a specific DNA sequence change or knock-in a gene, researchers must co-deliver a donor DNA template to trigger repair via:
(A) Homology-Directed Repair (HDR)
(B) NHEJ
(C) Nucleotide Excision Repair
(D) Photoreactivation

2015
**233.** Unlike NHEJ, Homology-Directed Repair (HDR) occurs almost exclusively during which phases of the cell cycle?
(A) $G_0$ and $G_1$
(B) Late S and $G_2$
(C) Prophase and Metaphase
(D) Anaphase and Telophase

2025
**234.** In genome editing, the "protospacer" refers to the:
(A) DNA repair template
(B) Targeted region of the host genomic DNA
(C) Nuclease protein
(D) Sequence between two genes

2014
**235.** The fundamental goal of creating a targeted gene knockout in a livestock embryo is usually achieved by inducing a frameshift mutation that introduces a:
(A) Premature Stop Codon (PTC)
(B) Missense mutation
(C) Splice variant
(D) Synonymous mutation

2015
**236.** The unintended cleavage of DNA at genomic loci with sequences similar to the target site is termed an:
(A) On-target effect
(B) Epigenetic silencing
(C) OfF-target effect
(D) Imprinting error

2022
**237.** Microinjection for generating edited livestock models typically introduces genome-editing reagents directly into the:
(A) Cytoplasm of a somatic cell
(B) Uterus of the surrogate
(C) Sperm head
(D) Zygote's pronucleus

2015
**238.** Somatic Cell Nuclear Transfer (SCNT) is often combined with genome editing in livestock to ensure that the resulting animal is:
(A) Non-transgenic
(B) Polyploid
(C) Fully clonal and uniformly edited, avoiding mosaicism
(D) Completely free of mutations

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2008
**239.** Mosaicism in founder genome-edited animals occurs when the nuclease remains active past the:
(A) One-cell embryo stage
(B) Blastocyst stage
(C) Gastrulation stage
(D) Birth of the animal

2011
**240.** The efficiency of HDR in mammalian embryos compared to NHEJ is generally:
(A) Significantly higher
(B) Identical
(C) Significantly lower
(D) 100% in all cells

2002
**241.** Meganucleases (homing endonucleases) were the first programmable genome editing tools. They occur naturally in microbial genomes and recognize target DNA sequences of approximately:
(A) 3 to 6 bp
(B) 14 to 40 bp
(C) 100 bp
(D) 500 bp

2017
**242.** The primary limitation restricting the widespread use of meganucleases in livestock engineering is:
(A) Limitation to RNA cleavage exclusively
(B) Induction of massive host immune responses
(C) Complex structural recognition constraints
(D) Induction of lethal chromosomal shattering

2008
**243.** Zinc Finger Nucleases (ZFNs) represent the first major class of engineered artificial nucleases. They are formed by fusing zinc finger DNA-binding domains to the cleavage domain of:
(A) FokI
(B) Cas9
(C) EcoRI
(D) BamHI

2003
**244.** A single standard zinc finger motif recognizes and binds to exactly how many base pairs of DNA?
(A) 1 bp
(B) 3 bp
(C) 5 bp
(D) 10 bp

2015
**245.** Because the FokI nuclease domain must dimerize to cleave double-stranded DNA, ZFNs must be engineered and delivered as:
(A) A single monomer
(B) A pair (Left and Right ZFNs)
(C) A tetramer
(D) An RNA-protein complex

2006
**246.** To design a ZFN pair targeting a unique 18 bp sequence on one strand, the corresponding zinc finger array would require how many individual zinc finger motifs?
(A) 3
(B) 6
(C) 9
(D) 18

2010
**247.** The FokI cleavage domain generates DNA breaks that feature:
(A) Blunt ends
(B) 3' overhangs
(C) Single-stranded nicks only
(D) 5' overhangs (sticky ends)

2002
**248.** A significant drawback of early ZFN technology was "context dependence," meaning:
(A) The binding specificity of one zinc finger was influenced by its neighboring fingers
(B) The nucleases only worked in certain tissues
(C) They required a specific temperature to function
(D) They only recognized heavily methylated DNA

2005
**249.** Which metal ion is structurally required to coordinate the protein folding of the classical Cys2-His2 zinc finger motif?
(A) Iron ($Fe^{2+}$)
(B) Zinc ($Zn^{2+}$)
(C) Magnesium ($Mg^{2+}$)
(D) Calcium ($Ca^{2+}$)

2015
**250.** Before CRISPR, ZFNs were successfully used to generate the first knockout pigs lacking the α-1,3-galactosyltransferase ($GGTA_1$) gene. This modification primarily aimed to:
(A) Increase muscle mass
(B) Prevent hyperacute rejection in xenotransplantation
(C) Improve resistance to PRRS
(D) Make the pigs hornless

2002
**251.** OfF-target cleavage by ZFNs is frequently caused by:
(A) Formation of active FokI homodimers at solitary binding sites
(B) Formation of heterodimers between mismatched Left and Right nucleases
(C) RNA binding instead of DNA
(D) Spontaneous FokI cleavage without DNA binding

2024
**252.** To reduce ZFN ofF-target effects, researchers engineered "obligate heterodimer" FokI domains, ensuring that cleavage only occurs when:
(A) The two domains are identical
(B) Zinc is absent from the reaction
(C) Two distinct, uniquely engineered FokI variants bind adjacent to each other
(D) The target DNA is single-stranded

2006
**253.** TALENs stand for Transcription Activator-Like Effector Nucleases. The TALE DNA-binding domains were originally discovered in:
(A) Mammalian immune cells
(B) Archaea living in hydrothermal vents
(C) Bacteriophages
(D) Plant pathogenic bacteria of the genus Xanthomonas

2017
**254.** A single TALE repeat domain consists of approximately 34 amino acids and uniquely recognizes:
(A) 1 base pair of DNA
(B) 3 base pairs of DNA
(C) A specific RNA sequence
(D) Methylated cytosines only

2016
**255.** The DNA base recognition specificity of a TALE repeat is dictated exclusively by two hypervariable amino acids located at positions 12 and 13, known as the:

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(A) Protospacer Adjacent Motif (PAM)
(B) Repeat-Variable Diresidue (RVD)
(C) Zinc coordination site
(D) Leucine zipper

2022
**256.** If a TALE RVD consists of the amino acids Asn-Ile (NI), it specifically recognizes which nucleotide?
(A) Adenine (A)
(B) Cytosine (C)
(C) Guanine (G)
(D) Thymine (T)

2015
**257.** If a TALE RVD consists of His-Asp (HD), it specifically recognizes which nucleotide?
(A) Adenine (A)
(B) Cytosine (C)
(C) Guanine (G)
(D) Thymine (T)

2011
**258.** Like ZFNs, TALENs achieve double-strand DNA cleavage by utilizing the cleavage domain of:
(A) Cas9
(B) Cas12a
(C) FokI
(D) EcoRI

2012
**259.** A major advantage of TALENs over ZFNs is that the modular assembly of TALE arrays is:
(A) Completely free of context dependence, making design predictable
(B) Much smaller in protein size
(C) Unreliant on protein dimerization
(D) Directed by an RNA guide

2004
**260.** A structural requirement for natural TALE proteins binding to DNA is that the target sequence must universally begin with a:
(A) Guanine (G)
(B) Thymine (T)
(C) Cytosine (C)
(D) Adenine (A)

2022
**261.** The major technical challenge in constructing plasmids encoding TALEN arrays is:
(A) Finding a suitable FokI domain
(B) The highly repetitive nature of the DNA sequence encoding the TALE repeats, prone to recombination
(C) Delivering RNA to the cells
(D) Removing the PAM sequence

2009
**262.** Recombinetics famously used TALEN technology in 2016 to introduce a mutation into dairy cattle to produce the "polled" (hornless) phenotype. They targeted which specific locus?
(A) MSTN
(B) $DGAT_1$
(C) POLLED (PC) locus on chromosome 1
(D) PRNP

2019
**263.** When designing a pair of TALENs, the "spacer" region between the left and right TALE binding sites is critical because it dictates:
(A) The binding of the single-guide RNA
(B) The speed of the ribosomes
(C) The RVD code
(D) The optimal distance for FokI dimerization and cleavage

2006
**264.** Although highly specific, a disadvantage of TALENs compared to CRISPR-Cas9 for large-scale genetic screening is:
(A) TALENs require engineering a completely new, large protein for every new DNA target
(B) TALENs can only cut RNA
(C) TALENs cannot be injected into embryos
(D) TALENs are lethal to eukaryotic cells

NODIA

2017
**265.** The CRISPR-Cas system evolved in bacteria and archaea as a natural mechanism of:
(A) Horizontal gene transfer
(B) Adaptive immunity against invading bacteriophages and plasmids
(C) Quorum sensing
(D) DNA replication initiation

2022
**266.** In the engineered Type II CRISPR-Cas9 system used for genome editing, target specificity is directed entirely by a:
(A) Zinc finger protein
(B) Single-guide RNA (sgRNA)
(C) TALE repeat array
(D) FokI homodimer

2014
**267.** The sgRNA is an artificial fusion of two natural CRISPR RNA components: the target-specific crRNA and the structural:
(A) tracrRNA
(B) mRNA

(B) Diverged from a single domestication event 10,000 years ago
(C) Originated from independent domestication centers of wild aurochs
(D) Cannot interbreed

2017
**173.** The primary reason mtDNA is superior to nuclear DNA for ancient DNA (aDNA) analysis of fossilized livestock bones is:
(A) It is perfectly stable and never degrades
(B) It contains long, unbroken repetitive elements
(C) It is easily amplified using RAPD primers
(D) The high copy number of mtDNA per cell improves recovery chances

2016
**174.** A distinct, monophyletic grouping within a phylogenetic tree based on inherited mtDNA variations is termed a:
(A) Genotype
(B) Serotype
(C) Haplogroup
(D) Phenotype

2010
**175.** Domestic sheep (Ovis aries) exhibit multiple highly distinct maternal lineages. These are typically designated as Haplogroups:
(A) A, B, C, D, and E
(B) $T_1$, $T_2$, $T_3$
(C) $I_1$, $I_2$, $I_3$
(D) $B_1$, $B_2$, $B_3$

2021
**176.** Which algorithm is commonly used to reconstruct livestock phylogenetic trees from aligned D-loop sequences?
(A) BLASTn
(B) Smith-Waterman
(C) Needleman-Wunsch
(D) Maximum Likelihood or Neighbor-Joining

2019
**177.** The substitution rate in the D-loop is often referred to as a "molecular clock." This clock allows researchers to estimate:
(A) The divergence time between distinct haplogroups
(B) The exact number of individuals in a founder population
(C) The phenotypic traits of the ancestor
(D) The recombination frequency of nuclear DNA

2019
**178.** Dog (Canis lupus familiaris) mtDNA sequencing definitively proved that domestic dogs originated entirely from the:
(A) Coyote
(B) Jackal
(C) Gray Wolf
(D) Dingo

2003
**179.** The Cytochrome b (cyt b) gene is sometimes sequenced alongside the D-loop. Compared to the D-loop, cyt b sequences are:
(A) More conserved and useful for deeper inter-species phylogeny
(B) Far more polymorphic and faster mutating
(C) Not translated into protein
(D) Found in the nuclear genome

2003
**180.** The domestication center for the taurine (Bos taurus) haplogroups (e.g., $T_1$, $T_2$, $T_3$) has been localized via mtDNA studies primarily to the:
(A) Indus Valley
(B) Andean highlands
(C) Central Asian steppes
(D) Fertile Crescent (Near East)

2010
**181.** RNA-Seq involves the high-throughput sequencing of which molecular pool?
(A) The entire genomic DNA
(B) The transcriptome (RNA transcripts)
(C) The proteome
(D) Small metabolites

2025
**182.** To selectively capture major messenger RNA (mRNA) from total eukaryotic RNA, most RNA-Seq library preparations utilize magnetic beads coated with:
(A) Random hexamers
(B) Anti-rRNA antibodies
(C) Oligo(dT) probes
(D) Adapter sequences

2025
**183.** Total RNA in a mammalian cell is primarily composed of:
(A) mRNA (approx. 80%)
(B) tRNA (approx. 80%)
(C) rRNA (approx. 80-90%)
(D) miRNA (approx. 50%)

2012
**184.** If transcriptomic analysis of non-polyadenylated RNAs (like circular RNAs or bacterial RNAs)

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(D) Retain the normal biological functions of the CD163 protein

2024

**280.** African Swine Fever Virus (ASFV) resistance is being researched by comparing domestic pigs to warthogs. A key gene target proposed for conferring tolerance is:
(A) RELA (p65 subunit of NF-κB)
(B) MSTN
(C) $DGAT_1$
(D) $GH_1$

2021

**281.** The generation of genome-edited cattle resistant to bovine tuberculosis targets genes involved in macrophage survival and immunity, such as:
(A) Beta-lactoglobulin
(B) NRAMP1 ($SLC11A_1$) or SP110
(C) Myostatin
(D) Stearoyl-CoA desaturase

2015

**282.** Transmissible Gastroenteritis Virus (TGEV) and Porcine Epidemic Diarrhea Virus (PEDV) enter host cells via aminopeptidase n (ANPEP). Editing this gene aims to confer resistance to:
(A) Respiratory diseases
(B) Enteric (intestinal) coronavirus diseases
(C) Neurological prions
(D) Skin lesions

2003

**283.** Avian Leukosis Virus (ALV) resistance in chickens can be engineered by deleting the viral receptor encoded by the:
(A) tvb gene
(B) B-G region of MHC
(C) MSTN gene
(D) Ovalbumin promoter

2001

**284.** Mad Cow Disease (BSE) in cattle is caused by misfolded prions. Complete knockout of which gene via genome editing prevents prion disease propagation?
(A) APP
(B) MAPT
(C) PRNP
(D) HTT

2023

**285.** In creating disease-resistant livestock, the strategy of knocking in a gene encoding a neutralizing antibody or an antiviral RNA interference (RNAi) cassette is considered:
(A) Targeted mutagenesis
(B) Epigenetic editing
(C) Base editing
(D) Transgenesis via genome editing

2023

**286.** Resistance to classical swine fever virus (CSFV) has been generated in pigs by inserting specific anti-CSFV small hairpin RNAs (shRNAs) targeted to the safe harbor locus:
(A) MSTN
(B) $GGTA_1$
(C) Rosa26
(D) CD163

2009

**287.** Which livestock species has been successfully genome-edited to lack the CD163 receptor, achieving full resistance to PRRSV infection in challenge trials?
(A) Cattle
(B) Pigs
(C) Sheep
(D) Goats

2006

**288.** A major regulatory advantage of generating disease-resistant animals via specific gene knockouts (like CD163 mutation) compared to traditional transgenesis is that they:
(A) Contain large quantities of foreign bacterial DNA
(B) Are sterile and cannot spread the trait
(C) Are classified as pharmaceutical drugs
(D) Do not contain any foreign DNA and mimic naturally occurring mutations

2004

**289.** Horns on dairy cattle present a severe injury risk to farm workers and other animals. The physical removal of horns (dehorning) causes immense stress. Genome editing provides a welfare-friendly alternative by introgressing the dominant:
(A) Celtic POLLED allele (Pc)
(B) Myostatin double-muscling allele
(C) Halothane mutation
(D) Callipyge mutation

NODIA

2002

**290.** The introgression of the POLLED trait into Holstein cattle via TALENs or CRISPR represents a major milestone because it avoids:
(A) Elimination of cloning technology usage
(B) Exemption from rigorous regulatory approval
(C) Avoidance of multi-decade backcrossing
(D) Elimination of the use of nucleases

**28.** (C) RNA polymerase III
SINEs, such as the Alu element, are derived from small cellular RNAs and are typically transcribed by the host enzyme RNA polymerase III. This polymerase is responsible for transcribing various non-coding RNAs, including tRNAs and 5S rRNA. The internal promoter sequences within SINEs allow them to be recognized by RNA polymerase III, facilitating the production of the RNA intermediates needed for retrotransposition.

**29.** (C) I, II, IV and V
Interspersed repeats like LINEs and SINEs make up a large portion of the mammalian genome and mobilize through RNA intermediates. SINEs depend on LINE machinery for movement, and both create target site duplications upon insertion. Alu elements specifically evolved from the 7SL RNA gene. These features collectively describe the biology and genomic impact of these mobile elements across various eukaryotic species.

**30.** (C) 7SL RNA
The Alu element, which is the most abundant SINE in the human genome, evolved from a specific cellular RNA. Its evolutionary ancestor is the 7SL RNA, which is a component of the signal recognition particle involved in protein secretion. Structural similarities and sequence homology confirm that Alu sequences originated from the retrotransposition and subsequent modification of this functional non-coding RNA molecule.

**31.** (B) Target Site Duplications (TSDs)
When a retrotransposon like a LINE or SINE integrates into a new genomic location, the repair of the staggered nicks in the host DNA creates short identical sequences. These sequences, which flank the newly inserted element, are called Target Site Duplications. They serve as a hallmark of transposition events. Their presence helps researchers identify and characterize the boundaries of mobile elements.

**32.** (D) tRNAs
In many mammalian genomes, including those of livestock, SINEs have evolved from different small RNA ancestors. While human Alu elements are derived from 7SL RNA, most SINEs in livestock species like cattle and sheep originated from transfer RNAs. These tRNA-derived SINEs share structural features with their ancestors, including internal promoters that allow for transcription by the host RNA polymerase.

**33.** (A) P-III, Q-I, R-IV, S-II
LINE-1 elements are autonomous non-LTR retrotransposons, while Alu elements are non-autonomous and rely on other elements for mobility. LTR retrotransposons are structurally similar to retroviruses, containing long terminal repeats. In contrast, DNA transposons move through a cut-and-paste mechanism without an RNA intermediate. Each of these mobile elements has distinct structural characteristics and mechanisms for increasing their presence within the genome.

**34.** (C) DNA methylation
To prevent genomic instability, cells employ several mechanisms to limit the activity of retrotransposons like LINEs. In mature somatic cells, the primary method for silencing these elements is DNA methylation at their promoter regions. This epigenetic modification prevents the transcription of the LINE RNA, thereby stopping the production of the enzymes needed for retrotransposition. This control is vital for maintaining integrity.

**35.** (A) Horizontal transfer from squamate reptiles
Bov-B is a long interspersed nuclear element that is highly prevalent in the genomes of cattle and other ruminants. Research has shown that Bov-B was likely introduced into the ruminant lineage through horizontal gene transfer from squamate reptiles, such as snakes and lizards. This rare event represents a significant horizontal movement of genetic material between distantly related vertebrate classes.

**36.** (C) 6.0 kb
A full-length, autonomous mammalian LINE-1 element contains a 5' untranslated region, two open reading frames, and a 3' untranslated region. The total length of such a functional element is approximately 6.0 kilobases. Most LINE-1 copies in the genome are truncated and inactive, but these full-length versions provide the necessary proteins for the mobilization of both themselves and non-autonomous SINEs.

**37.** (C) 60
The standard diploid chromosome number for cattle, belonging to the species Bos taurus, is 60. This includes 58 autosomes and two sex

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# ANSWERS

**1.** (A) Nucleosome
Nucleosomes consist of DNA wrapped around a histone octamer, forming a beads-on-a-string structure. This primary level of packaging organizes genomic DNA within the eukaryotic nucleus. Each unit includes approximately 147 base pairs of DNA and specific core histones. It represents the basic structural organization required for further compaction into higher-order chromatin fibers.

**2.** (C) $H_1$
The core histone octamer is composed of two subunits each of histones $H_2A$, $H_2B$, $H_3$, and $H_4$. Histone $H_1$ acts as a linker protein rather than a core component. It binds to the DNA between nucleosomes to facilitate higher-order folding. This protein plays a critical role in stabilizing the chromatin fiber and regulating gene expression levels.

**3.** (A) Both A and R are true and R is the correct explanation of A.
Euchromatin consists of loosely packed DNA that is accessible for transcription by RNA polymerase and other factors. Its open conformation allows regulatory proteins to bind efficiently to promoter regions. This state is associated with high transcriptional activity compared to the densely packed heterochromatin. Therefore, the structural accessibility directly correlates with the functional capacity for gene expression.

**4.** (D) 147 bp
The core nucleosome structure involves approximately 147 base pairs of DNA wrapped around the histone octamer. This specific length represents the DNA protected from enzymatic digestion within the core particle. While the total DNA per nucleosome including linkers can reach 200 base pairs, the core wrap itself is consistently observed at this shorter, precise length across various species.

**5.** (B) Solenoid structure
The solenoid structure represents the second level of DNA packaging, where nucleosomes are coiled into a fiber with a diameter of 30 nanometers. This arrangement provides a six-fold increase in DNA compaction compared to the 10 nm beads-on-a-string fiber. It is stabilized by linker histones and is a characteristic feature of interphase chromatin and further chromosomal organization.

NODIA

**6.** (C) Histone $H_3K_9$ methylation
Heterochromatin is characterized by dense DNA packaging and transcriptional silencing, often marked by specific histone modifications. Methylation of histone $H_3$ at lysine 9 is a hallmark epigenetic signal for this repressed state. These marks recruit proteins that further compact the chromatin, preventing access by the transcription machinery. Such regions often replicate late during the cell cycle phase.

**7.** (A) I and II
Nucleosome assembly is a highly regulated process starting with the deposition of an $H_3$-$H_4$ tetramer onto DNA, followed by the addition of two $H_2A$-$H_2B$ dimers. This sequence is facilitated by specialized histone chaperones like CAF-1. The assembly does not show an exclusive preference for GC-rich sequences. Instead, it occurs across the genome to organize the entire DNA strand.

**8.** (A) 30 nm fiber
The zig-zag model is a proposed structural arrangement for the 30 nm chromatin fiber. In this configuration, the linker DNA passes through the center of the fiber, connecting nucleosomes that are not immediately adjacent. This model contrasts with the solenoid model and explains how chromatin can be further compacted. It remains a fundamental concept in understanding higher-order genomic architecture.

**9.** (B) Constitutive heterochromatin
Constitutive heterochromatin remains in a highly condensed state throughout the cell cycle and is generally devoid of active genes. It is transcriptionally silent and typically replicates during the late stages of the S phase. These regions are often found near centromeres and telomeres. Their structural stability is vital for maintaining chromosomal integrity and regulating the overall nuclear organization.

abnormalities that might not be visible with standard G-banding or other techniques.

**47.** (B) Macrochromosomes and microchromosomes
The avian karyotype, including that of the chicken, is unique among vertebrates due to the presence of two distinct size classes of chromosomes. These include a few pairs of large macrochromosomes and numerous small, dot-like microchromosomes. Despite their small size, microchromosomes are gene-dense and important for the bird's genome. This organizational structure is a defining feature of the genomes of birds.

**48.** (D) ZW (heterogametic)
Birds utilize the ZW sex-determination system, which differs from the XY system found in mammals. In this system, the female is the heterogametic sex, possessing one Z and one W chromosome. Males are homogametic, carrying two Z chromosomes. This difference in sex determination has significant implications for the inheritance of sex-linked traits and the development of specialized breeding strategies in poultry.

**49.** (A) Variable Number Tandem Repeats (VNTRs)
Minisatellites are tandemly repeated DNA sequences where the repeat unit is typically between 10 and 60 base pairs long. They are also known as Variable Number Tandem Repeats, or VNTRs, because the number of repeats varies significantly between individuals. This high level of polymorphism made them the first markers used in DNA fingerprinting for individual identification and parentage testing in various species.

**50.** (A) 1 to 6 base pairs
Microsatellites, also known as Short Tandem Repeats or STRs, consist of very short DNA motifs that are repeated in tandem. The length of these repeat units typically ranges from one to six base pairs. Because the number of repetitions varies highly among individuals, microsatellites are excellent genetic markers for identification, linkage mapping, and studying population genetics in humans and livestock.

**51.** (D) I, II, III and IV
Microsatellites are highly valuable genetic markers because they are codominant and exhibit high levels of allelic polymorphism across the genome. They primarily mutate through a mechanism known as slipped strand mispairing during DNA replication. Their wide distribution and ease of analysis via PCR make them ideal for parentage testing, biodiversity studies, and mapping traits in diverse livestock populations and other organisms.

**52.** (D) Slipped strand mispairing
The primary mechanism for the high mutation rate and allelic variation in microsatellites is slipped strand mispairing. During DNA replication, the DNA polymerase may lose its place on the repetitive template, leading to the insertion or deletion of repeat units. This results in offspring having a different number of repeats than the parents, creating the high polymorphism observed at these loci.

**53.** (B) Subtelomeric regions
Minisatellites are not uniformly distributed throughout the genome but tend to cluster in specific areas. In many species, including humans and livestock, these sequences are heavily concentrated in the subtelomeric regions near the ends of chromosomes. Their location and high rate of recombination make them useful for studying genetic variation, although they have largely been replaced by microsatellites in modern applications.

**54.** (C) Codominant and highly polymorphic
Microsatellites are preferred for parentage testing in livestock because they are codominant, meaning both alleles in a heterozygote can be detected. They are also highly polymorphic, providing enough variation to distinguish between closely related individuals. These markers are easily amplified using PCR and analyzed with high precision. Their inheritance follows Mendelian principles, allowing for the clear exclusion or inclusion of parents.

**55.** (A) P-II, Q-I, R-IV, S-III
Minisatellites are classified as VNTRs, while microsatellites are STRs that often mutate through slipped strand mispairing. Dinucleotide repeats represent the most common type of microsatellite motif found in mammalian genomes. Telomeric repeats consist of specific TTAGGG arrays that protect the ends of chromosomes. Each of these tandem repeats has unique structural and functional roles that are exploited in genetic research.

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**19.** (A) Telomeres and centromeres
Highly repetitive DNA sequences, such as satellite DNA, are predominantly found in specific structural regions of the chromosome. These include the centromeres, which are essential for spindle attachment during division, and the telomeres, which protect chromosome ends. These regions are generally transcriptionally silent and consist of tandemly repeated units. Their presence is vital for maintaining chromosomal stability and proper segregation.

**20.** (D) Single-copy DNA
Genetic complexity refers to the total length of unique, non-repeating DNA sequences within a genome. Single-copy DNA possesses the highest genetic complexity because each sequence is unique and contains distinct information. In contrast, repetitive DNA, such as satellites or SINEs, has low complexity due to the frequent repetition of the same motifs. Therefore, protein-coding regions typically exhibit the greatest sequence diversity.

**21.** (C) Microsatellite → Minisatellite → Satellite
Tandem repeats are categorized based on the length of their basic repeat units. Microsatellites consist of the shortest motifs, typically one to six base pairs. Minisatellites have medium-length units, generally ten to sixty base pairs. Satellite DNA contains the longest repeat units, which can span hundreds of base pairs and form very large clusters. This hierarchy defines the different classes of repeats.

**22.** (C) Moderately repetitive interspersed sequences
Alu elements are short interspersed nuclear elements that are widespread in the human genome. They belong to the class of moderately repetitive DNA and move via a retrotransposition mechanism. These elements are approximately 300 base pairs long and do not encode their own mobilization enzymes. They are distributed throughout the genome and contribute significantly to genetic diversity and occasionally to disease.

**23.** (D) Single-copy
Pseudogenes are genomic sequences that resemble functional genes but have lost their ability to code for proteins due to mutations. Functional pseudogenes, which may have regulatory roles, are typically classified as single-copy DNA. This is because they usually occur in only one or a few locations, reflecting their origin from a unique gene through duplication or retrotransposition followed by inactivation.

**24.** (A) Satellite DNA
Satellite DNA consists of very long arrays of tandemly repeated sequences that can extend over hundreds of kilobases. These regions are a major component of constitutive heterochromatin and are predominantly found at centromeres and other heterochromatic blocks. Because these repeats are clustered together in large tandem arrays, they are distinct from interspersed repeats. They play essential roles in chromosomal structural integrity.

**25.** (B) Non-LTR retrotransposons
Long Interspersed Nuclear Elements, commonly known as LINEs, are a major class of retrotransposons found in eukaryotic genomes. They are specifically classified as non-LTR retrotransposons because they lack the long terminal repeats characteristic of retroviruses. LINEs mobilize through an RNA intermediate using a copy-and-paste mechanism. They represent a significant portion of the mammalian genome and contribute to its evolution.

**26.** (B) Reverse transcriptase and endonuclease
Functional LINE elements are autonomous retrotransposons that encode the proteins necessary for their own movement. These proteins include an endonuclease, which nicks the target DNA, and a reverse transcriptase, which copies the LINE RNA into new DNA at the insertion site. This enzymatic machinery allows LINEs to spread throughout the genome independently. These activities are essential for their "copy-and-paste" mobilization.

**27.** (A) Both A and R are true and R is the correct explanation of A.
Short Interspersed Nuclear Elements are non-autonomous because they lack the genes required to encode their own mobilization enzymes. They do not produce reverse transcriptase or endonuclease and must rely on the machinery provided by autonomous LINE elements to transpose. This parasitic relationship allows SINEs to replicate and spread throughout the genome despite their lack of functional coding sequences for transposition.

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**65.** (A) Offspring allele not in either parent
The principle of exclusion in parentage testing is based on the identification of alleles in the offspring that are not present in the suspected parent. If an offspring carries an allele that is not found in the alleged sire or dam, that individual can be definitively excluded as a biological parent. This method is highly reliable for narrowing down potential parentage in livestock populations.

**66.** (A) Restriction digestion → Gel electrophoresis → Southern transfer → Probe hybridization
Classical RFLP-based DNA fingerprinting follows a specific sequence of laboratory steps. First, the genomic DNA is cut into fragments using restriction enzymes. These fragments are then separated by size using gel electrophoresis. The DNA is transferred to a membrane via Southern blotting and finally detected using a specific probe through hybridization. This process reveals variations in fragment lengths between different individuals.

**67.** (A) Compatibility with PCR of degraded DNA
The transition from classical VNTR-based fingerprinting to STR profiling was driven by the advantages of PCR technology. STRs involve much shorter DNA fragments, which can be successfully amplified even from degraded or very small samples. This makes STR profiling more robust and versatile than RFLP, which requires large amounts of high-quality DNA. Consequently, STRs became the global standard for genetic identification.

**68.** (D) The number of loci analyzed
The power of discrimination refers to the ability of a genetic test to distinguish between two random individuals. This power increases exponentially as more independent loci are included in the analysis. By using a panel of multiple highly polymorphic STR markers, the probability of two individuals having the same genetic profile becomes extremely low. This makes the test highly effective for forensic and identification purposes.

**69.** (B) Amplifying multiple STR loci in a single PCR reaction
Multiplexing is a technique in DNA fingerprinting where multiple different target sequences, such as STR loci, are amplified in a single PCR reaction. This is achieved by using several pairs of primers, each labeled with different fluorescent dyes or designed to produce amplicons of different sizes. Multiplexing increases efficiency, reduces costs, and saves time by allowing many markers to be analyzed simultaneously.

**70.** (D) A is false but R is true.
While STR-based testing is extremely powerful, it is technically used to exclude individuals as parents rather than prove paternity with absolute certainty. By using a sufficient number of highly polymorphic markers, the probability of any other individual in the population sharing the same profile becomes negligible. Therefore, while it mathematically excludes others, it provides strong evidence for a specific sire.

**71.** (D) Microsatellites/SNPs
The International Society for Animal Genetics sets the standards for genetic testing in livestock to ensure consistency across laboratories. For cattle parentage verification, they recommend using standardized panels of microsatellites or Single Nucleotide Polymorphisms. These markers are selected for their high polymorphism and reliability. Using these standardized panels allows for the accurate comparison of genotypes and the global exchange of information.

**72.** (A) Migration time based on amplicon size
In capillary electrophoresis, PCR-amplified STR alleles are separated as they migrate through a narrow tube filled with a polymer. Different alleles at a single locus are distinguished by their migration time, which is directly related to the length of the DNA fragment. Smaller fragments move faster than larger ones. This allows for the precise measurement of allele sizes to the single-base level.

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**73.** (B) SNPs at restriction endonuclease sites
Restriction Fragment Length Polymorphism arises when mutations, such as SNPs, occur within the recognition sequence of a restriction enzyme. These mutations either create a new cutting site or destroy an existing one, resulting in fragments of different lengths when the DNA is digested. This variation is inherited and can be used as a marker for mapping genes and identifying genetic differences.

**74.** (B) Short arbitrary primers (typically 10-mers)
Random Amplified Polymorphic DNA is a PCR-based technique that uses short, arbitrary

chromosomes. Most of the autosomes in cattle are acrocentric, meaning the centromere is located very close to one end. Understanding the karyotype is essential for identifying chromosomal abnormalities and conducting genetic research in livestock breeding programs.

**38.** (C) Colchicine
Colchicine is a chemical agent commonly used in karyotyping to arrest cells during the metaphase stage of mitosis. It functions by inhibiting the polymerization of tubulin, which prevents the formation of the spindle apparatus. As a result, the chromosomes remain in their most condensed state and do not segregate. This allows for clear visualization and photographing of the individual chromosomes.

**39.** (A) I, II and III
Farm animals exhibit diverse diploid chromosome numbers. Domestic sheep have a $2n$ of 54, goats have 60, and pigs have 38. These numbers are standard for each species and are used in cytogenetic analysis to ensure health and fertility. The differences in chromosome count among these livestock reflect their distinct evolutionary histories and chromosomal rearrangements that occurred during their speciation.

**40.** (B) Metaphase
Metaphase is the stage of mitosis where chromosomes reach their maximum level of condensation. During this phase, they align at the cell's equatorial plate, making them clearly visible as individual units under a microscope. This high degree of compaction is ideal for karyotyping, as it allows for the precise identification of chromosomal structure, size, and banding patterns for genetic analysis.

**41.** (A) I, II, III, IV
Livestock species vary significantly in their diploid chromosome numbers. Swine have the lowest count among these examples at 38, followed by river buffalo with 50. Cattle possess 60 chromosomes, and chickens have the highest number at 78. This order from lowest to highest chromosome count reflects the standard karyotypes used in veterinary genetics and animal breeding research for these species.

**42.** (C) 64
The domestic horse, Equus caballus, has a standard diploid chromosome number of 64. This karyotype consists of 31 pairs of autosomes and one pair of sex chromosomes. Chromosomal analysis in horses is important for diagnosing fertility issues and studying evolutionary relationships within the equid family. The horse's chromosome count is distinct from other farm animals, such as cattle, sheep, and pigs.

**43.** (B) 50
The river buffalo, Bubalus bubalis, has a diploid chromosome number of 50. This distinguishes it from the swamp buffalo, which typically has 48 chromosomes. The difference is primarily due to a chromosomal translocation. Understanding these karyotypic variations is crucial for buffalo breeding programs and for studying the genetic diversity and evolutionary history of these important livestock animals across different regions.

**44.** (C) AT-rich and late replicating
G-banding is a widely used cytogenetic technique that involves treating chromosomes with trypsin followed by Giemsa stain. This process results in a pattern of dark and light bands. The dark G-bands correspond to genomic regions that are rich in Adenine and Thymine and typically replicate late. These bands generally contain fewer active genes compared to the light, Guanine-Cytosine rich regions.

**45.** (A) Both A and R are true and R is the correct explanation of A.
Robertsonian translocations occur when two acrocentric chromosomes fuse at their centromeres, creating a single metacentric or submetacentric chromosome. This process reduces the total diploid chromosome number because two chromosomes are replaced by one. Despite the change in count, the majority of the genetic material is preserved. This type of rearrangement is a significant mechanism for karyotype evolution in many livestock species.

**46.** (C) C-banding
C-banding is a specialized staining technique used in cytogenetics to highlight regions of constitutive heterochromatin. It predominantly stains the centromeres and other areas containing highly repetitive DNA sequences that remain condensed throughout the cell cycle. By revealing these specific regions, C-banding helps in identifying chromosomal variants and structural

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2006

**291.** Heat stress heavily impacts cattle productivity. Genome editing has been used to introduce the "slick" hair coat phenotype into thermointolerant breeds by targeting the:
(A) Prolactin receptor (PRLR) gene
(B) Growth hormone (GH) gene
(C) Insulin receptor (INSR) gene
(D) Keratin 14 ($KRT_14$) gene

2016

**292.** The slick coat mutation results in a significantly shorter, sparser hair coat and improved sweating, adapting high-yielding dairy breeds to:
(A) Sub-arctic climates
(B) High altitude, low-oxygen environments
(C) Intensive indoor stalling
(D) Tropical and subtropical climates

2023

**293.** Beta-lactoglobulin (BLG) is a major cow's milk protein that is absent in human milk and is a primary cause of:
(A) Lactose intolerance
(B) Milk allergy in human infants
(C) Mastitis in the cow
(D) Premature souring of milk

2006

**294.** Researchers successfully used genome editing to produce hypoallergenic cow's milk by knocking out the gene for:
(A) Casein alpha s1
(B) Lactalbumin
(C) Beta-lactoglobulin (BLG)
(D) Lactase

2016

**295.** To improve the nutritional profile of pork, pigs have been transgenic-edited with a fat-1 gene from C. elegans, allowing them to convert omega-6 fatty acids into:
(A) Trans fats
(B) Saturated fats
(C) Cholesterol
(D) Omega-3 fatty acids

2016

**296.** Piglets are highly susceptible to cold stress due to a lack of brown adipose tissue. CRISPR-Cas9 was utilized to reconstruct functional brown fat by knocking in a functional copy of the:
(A) UCP1 gene
(B) Leptin gene
(C) MSTN gene
(D) Insulin gene

2014

**297.** The knockout of the myostatin (MSTN) gene has been accomplished across multiple livestock species (cattle, pigs, sheep, goats) consistently resulting in:
(A) Dwarfism
(B) Significant muscle hypertrophy and increased meat yield
(C) Excessive fat deposition
(D) Loss of hair

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2013

**298.** To reduce phosphorus pollution in swine manure, which contributes to environmental eutrophication, "Enviropigs" were historically engineered to secrete which enzyme in their saliva?
(A) Amylase
(B) Phytase
(C) Cellulase
(D) Protease

2022

**299.** Preventing surgical castration in male pigs (welfare issue) while avoiding "boar taint" in meat has been experimentally approached by genome editing to disrupt puberty. The target gene typically knocked out is:
(A) $KISS_1$ or its receptor
(B) $DGAT_1$
(C) UCP1
(D) RYR1

2002

**300.** Xenotransplantation relies heavily on multiplex CRISPR-Cas9 editing of the pig genome to remove retroviruses and knockout multiple carbohydrate antigens, the most crucial of which is the alpha-gal epitope synthesized by the gene:
(A) CD163
(B) CMAH
(C) $GGTA_1$
(D) $B4GALNT_2$

**********

**56.** (A) Minisatellites
Unequal crossing over during meiosis is a major mutational driver for minisatellites. This process occurs when homologous chromosomes misalign at their repetitive regions, leading to an exchange of unequal amounts of DNA. As a result, one chromatid gains repeat units while the other loses them. This mechanism generates the significant variation in allele length that characterizes minisatellites and their use as markers.

**57.** (B) Dinucleotide
Among the various types of microsatellites, dinucleotide repeats are the most abundant and widely distributed in livestock genomes. These motifs consist of two alternating nucleotides, such as Cytosine and Adenine (CA), repeated multiple times. Because of their high frequency and high level of polymorphism, dinucleotide microsatellites have been extensively used as genetic markers for mapping, parentage verification, and population studies.

**58.** (B) Capillary electrophoresis
To analyze microsatellite markers, researchers use PCR to amplify the specific repetitive regions. The resulting DNA fragments are then resolved and sized with high precision using capillary electrophoresis. This technique separates the amplicons based on their length, allowing for the identification of different alleles. Fluorescently labeled primers enable automated detection, making this approach efficient for high-throughput genotyping in animal breeding programs.

**59.** (A) Codominant markers
Codominant markers, such as microsatellites, allow for the clear identification of both alleles present at a specific locus. This means that a heterozygote, which has two different alleles, produces two distinct signals that can be distinguished from a homozygote. This property is highly advantageous in genetic analysis, as it provides more information about the individual's genotype than dominant marker systems.

**60.** (C) Mismatch repair
Microsatellite instability refers to a condition where the number of repeat units at these loci changes rapidly due to errors in DNA replication. This is primarily caused by defects in the cellular mismatch repair system, which is responsible for correcting errors like slipped strand mispairing. When this repair pathway is compromised, mutations accumulate in repetitive sequences, serving as a marker for certain diseases.

**61.** (D) Alec Jeffreys
Alec Jeffreys pioneered the concept and methodology of DNA fingerprinting in 1984. He discovered that certain regions of the genome, known as minisatellites, contained sequences that were highly variable between individuals. By using probes to detect these repeats, he developed a method to create a unique genetic profile for each person. This breakthrough revolutionized forensic science, paternity testing, and animal genetics.

**62.** (C) Core sequences of minisatellites
The original DNA fingerprinting method developed by Alec Jeffreys used radioactive probes to target the core sequences found in minisatellites. These core sequences were common to many different minisatellite loci across the genome. When hybridized to restricted DNA, the probes produced a complex pattern of bands that was unique to each individual. This multi-locus approach provided a highly powerful tool for identification.

**63.** (C) I, II, III, IV and V
Modern DNA fingerprinting in livestock utilizes multiplex PCR to amplify multiple Short Tandem Repeat loci simultaneously. This approach requires very small amounts of DNA and generates precise, codominant genotype data. Fluorescent tags allow for automated sizing of alleles with high accuracy. These systems are powerful enough to resolve parentage disputes with extremely high probability, making them standard in the animal breeding industry.

**64.** (D) Inherit one allele from each parent
In parentage testing using microsatellite markers, the inheritance of alleles follows Mendelian rules. An offspring must inherit one allele from its biological mother and one allele from its biological father at every locus. By comparing the genotypes, researchers can confirm if a suspected parent could have contributed one of the alleles found in the offspring. This principle is fundamental to parentage verification.

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**10.** (B) Evict or reposition nucleosomes
Chromatin remodeling complexes use energy derived from ATP hydrolysis to alter the position or composition of nucleosomes. These complexes can slide nucleosomes along the DNA, evict them entirely, or exchange histone variants. This activity is essential for providing transcription factors and repair machinery access to the DNA template. It plays a central role in regulating gene expression and maintenance.

**11.** (B) P-IV, Q-I, R-II, S-III
Linker DNA serves as the connecting sequence between adjacent nucleosomes, while the $H_1$ histone stabilizes the nucleosome by sealing the DNA wrap. Scaffold proteins are essential for organizing chromatin into large loops, providing structural support to the chromosome. Telomeres act as protective caps at the ends of linear chromosomes. These components work together to maintain the integrity of genomic DNA.

**12.** (C) AT
Scaffold Attachment Regions are genomic sequences that mediate the binding of chromatin loops to the nuclear matrix or chromosomal scaffold. These regions are characteristically rich in Adenine and Thymine nucleotides. Their AT-rich nature facilitates the structural organization of chromosomes into functional domains. These attachments are crucial for regulating DNA replication, transcription, and overall chromosome stability within the cell.

**13.** (D) Structural and functional proteins
Single-copy DNA sequences are present in only one or a few copies per haploid genome. This class of DNA contains the majority of the structural and functional genes that encode proteins. Unlike repetitive DNA, single-copy sequences provide the specific instructions for synthesizing cellular enzymes and structural components. They represent the most genetically complex portion of the genome in eukaryotes.

**14.** (C) Moderately repetitive DNA
In mammalian genomes, moderately repetitive DNA constitutes a significant fraction of the total sequence. This class includes interspersed elements like LINEs and SINEs, as well as gene families for ribosomal RNA and transfer RNA. These sequences play significant roles in genome evolution, regulation, and structural organization. Their prevalence distinguishes the architecture of complex eukaryotic genomes from simpler prokaryotic ones.

**15.** (B) At the fastest rate
Reassociation kinetics, often analyzed via Cot curves, demonstrate that highly repetitive DNA sequences reanneal much faster than single-copy sequences. This occurs because the high frequency of these repeats increases the probability of finding a complementary strand during the hybridization process. The speed of reassociation is directly proportional to the number of copies of a sequence present within the genome.

**16.** (D) Genes for rRNAs and tRNAs
Moderately repetitive DNA sequences are found in multiple copies throughout the genome and often serve essential cellular functions. This category includes the genes encoding ribosomal RNAs and transfer RNAs, which are needed in high quantities for protein synthesis. Unlike highly repetitive satellite DNA, these sequences are often transcribed. They are distributed either in tandem clusters or interspersed throughout the genome.

**17.** (D) I, II, III and IV
The eukaryotic genome consists of several DNA classes with distinct properties. Highly repetitive DNA reassociates the fastest and is primarily non-coding, often forming satellite bands in density gradients. Moderately repetitive DNA includes interspersed elements like SINEs. All these characteristics accurately describe the complexity and organization of genomic sequences. These classifications are fundamental to understanding how genetic information is inherited.

**18.** (C) Base composition (GC content)
Satellite DNA is composed of highly repetitive sequences that often have a distinct base composition compared to the rest of the genome. During density gradient centrifugation, these differences in Guanine-Cytosine content cause satellite DNA to form separate bands from the bulk genomic DNA. This physical separation allows researchers to isolate these repetitive regions, which are primarily located at the centromeres.

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primers, typically ten nucleotides in length. These primers bind to multiple random sites across the genome. If two binding sites are close enough and in the correct orientation, the intervening DNA is amplified. Variations in the presence or absence of these binding sites result in polymorphic banding patterns between individuals.

**75.** (B) I and III
RFLP is a codominant marker system, meaning it can distinguish between homozygotes and heterozygotes. In contrast, RAPD is a dominant system and does not require prior sequence knowledge. AFLP involves a more complex process of restriction digestion followed by the ligation of specific adapters to the fragments. Each of these marker types has different requirements and provides varying levels of information.

**76.** (C) Low reproducibility between laboratories
A major technical disadvantage of RAPD markers is their low reproducibility. Because the primers are short and the annealing temperatures are low, the resulting banding patterns can vary significantly between different laboratories or even between different PCR runs. This sensitivity to experimental conditions makes it difficult to standardize RAPD data, limiting its use in large-scale genetic studies and long-term breeding programs.

**77.** (D) RFLP and PCR
Amplified Fragment Length Polymorphism combines the strengths of restriction digestion and PCR. In this method, genomic DNA is digested with enzymes, and adapters are ligated to the ends. Specific fragments are then selectively amplified using primers that match the adapters but have additional arbitrary bases. This provides the high reliability of RFLP with the efficiency and sensitivity of PCR-based analysis.

**78.** (C) RAPD
RAPD markers are considered dominant markers because the presence of a band indicates that at least one allele is present, while the absence of a band indicates the lack of that allele. This means that a heterozygote cannot be distinguished from a dominant homozygote, as both will produce a band. This limitation reduces the amount of genetic information obtained compared to codominant systems.

**79.** (A) P-IV, Q-I, R-II, S-III
RFLP markers are traditionally detected using Southern blotting, while RAPD utilizes arbitrary ten-mer primers for amplification. AFLP involves the use of restriction enzymes and the ligation of adapters to the resulting fragments. Single Nucleotide Polymorphisms represent variations at a single base position. These markers differ in their methodology and the type of genetic variation they capture within the genome for animal research.

**80.** (D) One rare cutter (e.g., EcoRI) and one frequent cutter (e.g., MseI)
The standard AFLP protocol employs a double digestion of genomic DNA using a combination of two restriction enzymes. Typically, one enzyme is a rare cutter, such as EcoRI, and the other is a frequent cutter, like MseI. This combination produces a large number of fragments of various sizes, which are then used for selective amplification and analysis in genetic studies.

**81.** (A) Arbitrary nucleotides at the 3' primer end
Selective amplification in AFLP is achieved by using primers that are complementary to the adapter and restriction site sequences but have one to three additional nucleotides at their 3' ends. These extra bases act as filters, allowing only the fragments with matching internal sequences to be amplified. This step reduces the complexity of the final banding pattern, making it easier to analyze genomic variation.

**82.** (C) Classical RFLP
Classical RFLP analysis requires a substantial amount of high-quality, high-molecular-weight genomic DNA. This is because the process involves digestion by restriction enzymes followed by Southern blotting, which is not an amplification-based technique. In contrast, PCR-based methods like RAPD, microsatellites, and SNP arrays can function with much smaller quantities of DNA, as they include steps to amplify target sequences.

**83.** (A) High band polymorphism without sequencing
AFLP provides a powerful tool for genetic analysis because it can detect a high number of polymorphic bands in a single assay without the need for prior sequence information. This

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**102.** (C) Linkage at a specific recombination fraction versus independent assortment
The LOD score is a statistical test used to determine the likelihood of linkage between two genetic loci. It calculates the logarithm of the ratio of two probabilities: the probability that the loci are linked at a specific recombination fraction versus the probability that they are unlinked and assorting independently. This score helps researchers decide if their mapping data support linkage.

**103.** (C) 1,000 times greater than independent assortment
A LOD score of 3.0 is a standard threshold used to establish significant evidence for genetic linkage. Since the LOD score is a base-10 logarithm, a value of 3.0 means that the likelihood of the observed data occurring due to linkage is 1,000 times greater than the likelihood of it occurring by chance through independent assortment. This level provides high statistical confidence.

**104.** (C) Genetically unlinked
When two loci show a recombination frequency of 50%, they are considered to be genetically unlinked. This occurs because the alleles at these loci assort independently during meiosis, just as if they were on different chromosomes. Even if two genes are on the same chromosome, they will appear unlinked if they are far enough apart that crossovers occur between them frequently.

**105.** (B) Multiple and unobserved crossover events
Haldane's mapping function is used to convert observed recombination frequencies into genetic distances in centimorgans. This mathematical correction accounts for the possibility of multiple crossovers occurring between two distant loci, which are not directly observable. By assuming that crossovers occur independently along the chromosome, the function provides a more accurate estimate of the true genetic distance than the raw recombination frequency.

**106.** (B) HalF-sib family designs
In dairy cattle, linkage studies often utilize a halF-sib family design. This structure involves large groups of offspring that share a single common sire but have different dams. Because dairy bulls can have thousands of progeny, this design provides a powerful way to observe recombination events and map quantitative trait loci. It is a highly efficient approach for livestock genetic analysis.

**107.** (A) Crossover interference
Kosambi's mapping function is an alternative to Haldane's that incorporates the biological phenomenon of crossover interference. Interference means that the occurrence of one crossover reduces the probability of another crossover happening nearby. By accounting for this effect, Kosambi's function often provides a more realistic estimate of genetic distance for many species, reflecting the non-random distribution of recombination events along chromosomes.

**108.** (C) X-ray induced chromosomal breaks and somatic cell fusion
Radiation hybrid mapping is a technique used to create high-resolution physical maps of genomes. It involves exposing donor cells to X-rays to break the chromosomes into fragments, which are then fused with rodent cells. By analyzing which donor markers are consistently retained together in different hybrid lines, researchers can determine the order and physical distance between loci, bypassing the need for meiosis.

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**109.** (D) In strong linkage disequilibrium with Quantitative Trait Loci (QTL)
Marker-Assisted Selection is a breeding strategy that uses DNA markers to select for desirable traits. It relies on the markers being in strong linkage disequilibrium with Quantitative Trait Loci that influence the trait. By selecting individuals that carry the favorable marker alleles, breeders can indirectly select for the underlying beneficial genes, even if the exact causal mutations are not yet known.

**110.** (C) Low heritability, sex-limitation, or requiring late-life measurement
Marker-Assisted Selection is particularly valuable for traits that are difficult or expensive to measure traditionally. This includes traits with low heritability, those that are sex-limited like milk production, or traits that are only expressed late in life or after slaughter. By using genetic markers, breeders can identify superior animals at a very young age, significantly accelerating the rate of genetic gain.

different impacts on the structure and function of the resulting DNA and proteins.

**93.** (A) Both A and R are true and R is the correct explanation of A.
SNPs have largely replaced microsatellites in livestock breeding because they are biallelic, highly abundant, and occur at a very high density across the entire genome. These characteristics make them exceptionally easy to automate for high-throughput genotyping. Because they can be analyzed efficiently on a large scale, SNPs are ideal for genomic selection, which requires markers covering all genetic regions within the population.

**94.** (B) 1%
In population genetics, a SNP is generally considered informative if its Minor Allele Frequency is at least 1%. This means that the less common allele must be present in at least 1% of the population. Loci with a very low MAF are often excluded from genome-wide studies because they lack sufficient variation to be statistically powerful for identifying associations with traits.

**95.** (B) Non-synonymous
A non-synonymous SNP is a variation within a coding region that results in a change to the amino acid sequence of the encoded protein. This type of mutation can potentially alter the protein's structure, stability, or activity, thereby influencing the organism's phenotype. In contrast, synonymous SNPs do not change the amino acid and are often considered to be functionally neutral in most biological contexts.

**96.** (D) Linkage disequilibrium with causative quantitative trait loci (QTL)
Genome-wide association studies use high-density SNP arrays to find markers that are linked to quantitative trait loci. This relies on linkage disequilibrium, where specific SNP alleles are consistently inherited together with the causative mutation. By identifying these associations, researchers can pinpoint genomic regions that influence important traits, even if the actual functional mutation itself has not yet been directly identified.

**97.** (A) Centimorgans (cM)
Genetic linkage mapping uses centimorgans as the standard unit to measure the distance between loci. One centimorgan represents a 1% probability that a recombination event will occur between two markers during meiosis. Unlike physical maps that use base pairs, genetic maps are based on the frequency of crossovers, which can vary across different regions of the chromosome and between different sexes.

**98.** (B) 1%
A centimorgan is a unit of genetic distance defined by the frequency of recombination. Specifically, two loci are one centimorgan apart if there is a 1% chance that they will be separated by a crossover event during a single generation. This measurement helps researchers estimate the relative order and distance between genes on a chromosome based on observed inheritance patterns in families.

**99.** (C) I, II, III and IV
Genetic mapping involves both physical maps measured in base pairs and linkage maps based on meiotic recombination. Linkage analysis utilizes crossover events to order markers, and the LOD score provides a statistical measure of how likely two loci are linked. Multiple crossovers can lead to an underestimation of distance, so mathematical functions are used to correct for these unobserved events.

**100.** (D) Base pairs (bp)
Physical genome maps represent the actual distance between genetic markers or genes along a DNA molecule. These distances are measured in base pairs, kilobases, or megabases. Unlike genetic maps, which are based on recombination frequencies, physical maps provide a direct measurement of the DNA sequence. This information is essential for sequencing projects and for identifying the exact location of functional genomic elements.

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**101.** (A) P-IV, Q-III, R-I, S-II
Physical maps measure the actual base pair distance between loci, while genetic maps are based on meiotic crossover rates. The recombination frequency reflects the proportion of non-parental gametes produced during meiosis. A LOD score is a statistical tool used to assess the likelihood that two markers are linked. Together, these concepts allow researchers to build comprehensive models of genome organization and inheritance.

**140.** (C) Highly compact with no Class III region
The chicken B-complex is significantly more compact than the MHC regions found in mammals. It lacks many of the non-essential genes and the extensive Class III region found in species like humans or cattle. Despite this minimalist structure, it contains all the necessary components for effective antigen presentation and is strongly linked to disease resistance, particularly against the Marek's disease virus.

**141.** (D) The alpha-1 and beta-1 domains of two distinct chains
The peptide-binding cleft of an MHC Class II molecule is an open-ended groove formed by the interaction of two different polypeptide chains, the alpha and beta chains. Specifically, the alpha-1 and beta-1 domains from these two chains come together to create the site where the antigenic peptide is held. This structure allows Class II molecules to bind longer peptides than Class I.

**142.** (C) Complement system and inflammatory cytokines
The Class III region of the MHC is a gene-dense area that contains a variety of genes with diverse functions in the immune system. Notable among these are genes encoding components of the complement system, which helps clear pathogens, and several inflammatory cytokines, such as tumor necrosis factor. These proteins play important roles in the innate immune response and the coordination of inflammation.

**143.** (B) PCR-sequence based typing (SBT)
Modern typing of MHC alleles in livestock, such as SLA in pigs or BoLA in cattle, primarily relies on PCR-sequence based typing. This method involves amplifying specific, highly polymorphic exons of the MHC genes and then sequencing them to identify the precise allele present. This high-resolution approach provides much more detail than older serological methods, allowing for more accurate disease studies.

**144.** (D) Heterozygote advantage and frequency-dependent selection
The high polymorphism of MHC genes is maintained by balancing selection, including mechanisms like heterozygote advantage and frequency-dependent selection. Individuals that are heterozygous at MHC loci can present a wider variety of peptides, potentially providing better protection against a broader range of pathogens. This evolutionary pressure prevents any single allele from becoming dominant, ensuring the genetic diversity of the immune system.

**145.** (A) CD4+ T helper cells
MHC Class II molecules are responsible for presenting peptides derived from exogenous antigens, which are taken up from outside the cell by endocytosis or phagocytosis. These peptides are then displayed to CD4+ T helper cells. This interaction is critical for activating the T cells, which in turn stimulate B cells to produce antibodies and help macrophages destroy the ingested pathogens.

**146.** (C) Invariant chain (Ii)
To ensure that MHC Class II molecules only bind to peptides in the endosomal compartment, they are initially assembled in the endoplasmic reticulum with a protein called the invariant chain. The invariant chain blocks the peptide-binding groove, preventing the binding of endogenous peptides. Once the complex reaches the endosome, the invariant chain is degraded, allowing the molecule to pick up exogenous antigens.

**147.** (A) Proteasome
Proteins produced within the cell, including viral proteins during an infection, are degraded into small peptides by a large multi-protein complex called the proteasome. These peptides are then eligible for presentation on MHC Class I molecules. This degradation pathway is a continuous process that allows the immune system to monitor the internal environment of the cell for any signs of infection.

**148.** (D) TAP1/TAP2 transporter
After being generated in the cytoplasm by the proteasome, peptides are moved into the lumen of the endoplasmic reticulum by the TAP transporter. This transporter, composed of two subunits called TAP1 and TAP2, is an ATP-dependent pump. Once inside the ER, the peptides are loaded onto newly synthesized MHC Class I molecules with the help of various chaperone proteins for presentation.

**149.** (B) Bovine Leukemia Virus (Enzootic Bovine Leukosis)
Research has demonstrated that specific alleles of the BoLA system in cattle are linked to

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**111.** (B) Genomic region strongly associated with variation in a continuously distributed trait
A Quantitative Trait Locus is a genomic region that is statistically associated with variation in a complex, continuously distributed trait, such as growth rate or milk yield. These traits are typically influenced by multiple genes and environmental factors. Identifying QTLs allows researchers to understand the genetic basis of important performance characteristics and provides targets for marker-assisted selection in livestock breeding.

**112.** (D) The density of markers and the size of the mapping population
The ability to accurately map a QTL to a small genomic interval is primarily limited by the density of the markers used and the size of the population being studied. A larger number of individuals and higher marker density provide more recombination events and better resolution. Improving these factors is essential for narrowing down a QTL to the specific gene or mutation.

**113.** (A) The proportion of phenotypic variance explained by the marker genotype
In marker-assisted selection, the marker effect refers to the amount of phenotypic variation for a specific trait that can be attributed to the different genotypes at a marker locus. This value helps breeders understand how much improvement can be expected by selecting for that marker. It is a measure of the association strength between the marker and the underlying QTL.

**114.** (C) Marker-assisted backcrossing (MABC)
Marker-assisted backcrossing is a technique used to introgress a specific, desirable allele from one breed into another. By using markers to select for the target gene and against the rest of the donor's genome, breeders can rapidly recover the recipient breed's characteristics while retaining the new trait. This approach is much faster and more precise than traditional backcrossing methods.

**115.** (A) Variable linkage phase across families
A significant challenge in early MAS programs was the variation in linkage phase between different families. This meant that the favorable allele of a QTL might be linked to different marker alleles in different individuals. As a result, the association between the marker and the trait had to be determined for each family separately, making large-scale implementation complex and labor-intensive.

**116.** (D) Quantitative Trait Nucleotide (QTN) or causal mutation
Fine mapping is the process of narrowing down a large QTL region to a much smaller interval, ideally identifying the specific Quantitative Trait Nucleotide or causal mutation. This requires using a very high density of markers and a large number of individuals to capture infrequent recombination events. Identifying the exact mutation is the ultimate goal for understanding the biology of traits.

**117.** (B) The estimated recombination frequencies between adjacent flanking markers
Interval mapping is a statistical method for QTL detection that analyzes the entire genomic interval between two flanking markers. By using the recombination frequencies between the markers, this approach provides a more accurate estimate of the QTL position and its effect than testing markers one at a time. It is a foundational technique for building genetic maps and identifying important traits.

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**118.** (C) Genomic Selection (GS)
Genomic Selection is an advanced breeding method that uses a large set of markers distributed across the entire genome to estimate the breeding value of individuals. Unlike MAS, which focuses on a few major QTLs, genomic selection captures the effects of all genes simultaneously. This approach has transformed livestock breeding by increasing the accuracy of selection and reducing generation intervals significantly.

**119.** (D) Multiple testing of thousands of markers
When conducting QTL analysis with thousands of markers, there is a high risk of finding false associations by chance. To address this, researchers apply genome-wide significance thresholds to correct for multiple testing. This ensures that only the most robust associations are reported as significant. These corrections are essential for maintaining the scientific validity and reliability of large-scale genetic mapping studies.

makes it more efficient than classical RFLP and more reliable than RAPD. AFLP is particularly useful for fingerprinting, mapping, and studying diversity in species where genomic resources are limited or not yet developed.

**84.** (D) RFLP
Restriction Fragment Length Polymorphism captures genetic variation that affects the size of DNA fragments produced by restriction enzymes. These variations can result from SNPs that alter a restriction site or from insertions and deletions that change the distance between two sites. By analyzing these fragment lengths, researchers can map genetic loci and identify variations associated with specific traits or diseases in animals.

**85.** (B) 500 to 1,000 bp
Single Nucleotide Polymorphisms are the most frequent type of genetic variation found in the genome. In mammalian species, they occur approximately once every 500 to 1,000 base pairs. Their high density and abundance make them incredibly powerful tools for fine-scale mapping of traits and for conducting genome-wide studies. They represent the smallest unit of genetic difference between individuals at sequence level.

**86.** (A) Diallelic (biallelic)
The majority of SNPs are biallelic, meaning that only two different nucleotides are found at that specific position within a population. While triallelic or tetra-allelic SNPs can occur, they are extremely rare. This biallelic nature simplifies the genotyping process and makes SNPs highly suitable for automated high-throughput analysis using arrays or sequencing, which is essential for modern livestock genomic selection.

**87.** (B) I and III
SNPs are valuable genetic markers because they are predominantly codominant and can be analyzed using automated high-throughput methods. Although they have lower individual mutation rates than microsatellites, their high density across the genome provides exceptional resolution for genetic studies. Synonymous SNPs do not change the amino acid sequence, whereas non-synonymous ones do, potentially affecting the function of the encoded protein.

**88.** (A) Transition
A transition is a type of single nucleotide substitution where a purine is replaced by another purine, such as Adenine for Guanine, or a pyrimidine is replaced by another pyrimidine, like Cytosine for Thymine. These mutations occur more frequently in the genome than transversions, which involve the replacement of a purine by a pyrimidine or vice versa. Transitions are common SNP variations.

**89.** (A) II, III, IV, I
High-density SNP genotyping begins with the amplification of the entire genome, followed by fragmentation and precipitation of the DNA. The fragments are then hybridized to specific oligonucleotide probes on a bead chip. Finally, an allele-specific single base extension step is performed to identify the nucleotide present at each SNP locus. This systematic process allows for the simultaneous analysis of thousands of markers.

**90.** (B) Sequence-specific nucleic acid hybridization
High-density SNP arrays operate on the principle of sequence-specific nucleic acid hybridization. Genomic DNA fragments are allowed to bind to complementary oligonucleotide probes that are fixed to a solid surface, such as a bead or a chip. The specificity of this binding, combined with detection methods like single-base extension, allows for the accurate determination of the genotype at many thousands of loci.

**91.** (A) Primer extension using fluorescent ddNTPs
Illumina BeadChip technology utilizes a single-base extension method to distinguish between different SNP alleles. After genomic DNA hybridizes to the probes on the beads, a DNA polymerase adds a single fluorescently labeled dideoxynucleotide that is complementary to the SNP position. The color of the fluorescence indicates which allele is present. This method is highly accurate and allows for massive parallel genotyping.

**92.** (C) Transversion
A transversion is a specific type of single nucleotide polymorphism where a purine base is substituted with a pyrimidine base, or vice-versa. For example, replacing a Cytosine with an Adenine is a transversion. These mutations are less common than transitions but are important sources of genetic variation. They can have

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**130.** (C) RYR1
The halothane test was a traditional method used to identify pigs carrying the RYR1 mutation. Pigs were exposed to halothane gas, and those with the mutation would exhibit characteristic muscle rigidity. This phenotypic screen allowed breeders to identify and remove carriers of the stress gene from their populations, helping to reduce the incidence of Porcine Stress Syndrome and improve meat quality.

**131.** (A) Muscle glycogen and meat quality
A mutation in the PRKAG3 gene, often referred to as the Rendement Napole gene, significantly affects the meat quality of Hampshire pigs. This mutation leads to an abnormally high accumulation of glycogen in the muscles. During the conversion of muscle to meat after slaughter, this glycogen is broken down into lactic acid, resulting in a lower pH and poor meat quality.

**132.** (D) Microsatellite linkage mapping
Weaver syndrome is a neurodegenerative disorder found in Brown Swiss cattle that is inherited as an autosomal recessive trait. Researchers identified the genomic region responsible for this defect by using microsatellite linkage mapping. By tracking the inheritance of specific markers in affected families, they were able to pinpoint the locus, enabling the development of genetic tests to manage carriers.

**133.** (B) BoLA
The Major Histocompatibility Complex is a crucial genomic region involved in the immune response of all vertebrates. In cattle, this complex is specifically known as the Bovine Lymphocyte Antigen system, or BoLA. It contains genes that encode proteins responsible for presenting antigens to T cells, playing a central role in disease resistance and the animal's overall ability to fight infections.

**134.** (C) Pigs
In pigs, the Major Histocompatibility Complex is referred to as the Swine Leukocyte Antigen system, or SLA. This region encodes several classes of molecules that are essential for the recognition of foreign antigens by the immune system. The high level of polymorphism in the SLA system is a key factor in how different individuals and breeds respond to various pathogens and vaccines.

**135.** (D) B-complex
The chicken Major Histocompatibility Complex is known as the B-complex. It was one of the first MHC systems to be studied in detail and is noted for its high level of association with resistance to various viral diseases. The organization of the chicken B-complex is more compact than the mammalian MHC, but it performs the same fundamental role in immune recognition.

**136.** (A) CD8+ Cytotoxic T cells
MHC Class I molecules are expressed on the surface of almost all nucleated cells. Their primary function is to present fragments of endogenous antigens, such as those from viruses or internal proteins, to CD8+ cytotoxic T cells. This allows the immune system to identify and destroy cells that are infected or otherwise abnormal, maintaining the health of the host organism.

**137.** (B) Beta-2 microglobulin
An MHC Class I molecule is composed of a polymorphic heavy chain and a non-polymorphic light chain called beta-2 microglobulin. Unlike the heavy chain, which is encoded within the MHC region, beta-2 microglobulin is encoded by a gene located on a different chromosome. It is essential for the stable folding and surface expression of the Class I complex on the cell.

**138.** (B) Professional antigen-presenting cells (e.g., dendritic cells, macrophages)
MHC Class II molecules have a more restricted distribution than Class I molecules. They are primarily expressed on professional antigen-presenting cells, such as dendritic cells, macrophages, and B lymphocytes. These molecules present antigens derived from extracellular pathogens to CD4+ T helper cells, which then coordinate the broader immune response, including the production of antibodies and activation of other immune cells.

**139.** (A) Polymorphism at peptide-binding clefts
The MHC region is famous for its extreme level of polymorphism, particularly in the parts of the genes that encode the peptide-binding cleft. This variation allows different individuals within a population to recognize and respond to a vast array of different pathogens. This diversity is maintained by evolutionary pressure, ensuring that at least some members of a population can survive new infections.

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**120.** (A) Type I error
In statistical testing for QTLs, a Type I error occurs when a researcher incorrectly concludes that a significant association exists when it does not. This is also known as a false positive. Controlling the rate of Type I errors is critical in genomic studies to avoid identifying incorrect genomic regions as being responsible for important traits, which could mislead breeding efforts.

**121.** (B) Milk fat percentage and yield
The $DGAT_1$ gene encodes an enzyme that plays a critical role in the synthesis of triglycerides. In dairy cattle, variations in this gene have been identified as a major factor influencing milk composition. Specifically, a well-known mutation in $DGAT_1$ is strongly associated with significant changes in milk fat percentage and total milk yield, making it an important target for selection.

**122.** (B) Lysine to Alanine (K232A)
A specific mutation in the $DGAT_1$ gene, where the amino acid Lysine is replaced by Alanine at position 232, is a major functional variation in cattle. This K232A substitution alters the enzyme's activity, leading to significant differences in milk fat synthesis. The Alanine variant is generally associated with lower fat content but higher overall milk volume compared to the Lysine variant.

**123.** (A) Belgian Blue
The Belgian Blue cattle breed is famous for a phenotype known as "double-muscling," which is characterized by an extreme increase in muscle mass. This trait is caused by mutations in the Myostatin gene that result in a non-functional protein. Because myostatin normally limits muscle growth, its absence leads to the uncontrolled proliferation of muscle fibers, resulting in very high meat yields.

**124.** (C) Inhibit myogenesis and muscle cell growth
Myostatin is a protein that acts as a negative regulator of skeletal muscle development. Its normal function is to inhibit the proliferation and differentiation of myoblasts, thereby controlling the overall growth of muscle tissue. Mutations that disrupt the myostatin gene lead to increased muscle mass, as the natural braking mechanism for muscle growth is lost, resulting in phenotypes like double-muscling.

**125.** (D) Ovulation rate and litter size
The FecB gene, also known as the Booroola gene, is a major gene that significantly affects the reproductive performance of sheep. A mutation in this gene leads to a dramatic increase in the ovulation rate and, consequently, a larger litter size. This trait is highly sought after in certain sheep breeds to improve the efficiency and output of lamb production.

**126.** (C) Bone Morphogenetic Proteins (BMPR1B)
The FecB mutation in sheep is located within the BMPR1B gene, which encodes a receptor for bone morphogenetic proteins. These proteins are members of the transforming growth factor-beta superfamily and are involved in regulating various cellular processes, including those in the ovary. The mutation alters the signaling pathway of these proteins, leading to increased follicular development and higher ovulation rates.

**127.** (B) Porcine Stress Syndrome (PSS) and Pale Soft Exudative (PSE) meat
A mutation in the RYR1 gene is the primary cause of Porcine Stress Syndrome in pigs. This condition makes animals highly susceptible to stress, which can lead to sudden death or the production of Pale Soft Exudative meat. PSE meat is of poor quality due to its reduced water-holding capacity and undesirable texture, causing significant economic losses in the pork industry.

**128.** (D) Calcium
The RYR1 gene encodes the ryanodine receptor, a large protein that functions as a calcium release channel in the sarcoplasmic reticulum of muscle cells. This channel is essential for the process of excitation-contraction coupling. In pigs with the RYR1 mutation, the channel becomes leaky, causing an excessive release of calcium ions, which leads to hypermetabolism and stress-related symptoms.

**129.** (A) Polar overdominance (imprinting)
The Callipyge mutation in sheep causes extreme muscle hypertrophy, particularly in the hindquarters. Its inheritance is unique and follows a pattern called polar overdominance, a form of genomic imprinting. In this case, only individuals that inherit the mutation specifically from their father show the phenotype. This complex genetic mechanism distinguishes Callipyge from typical recessive or dominant muscle-related mutations in livestock.

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the animal's response to the Bovine Leukemia Virus. Certain haplotypes are associated with resistance to the development of persistent lymphocytosis, a symptom of Enzootic Bovine Leukosis. Identifying these genetic associations helps in understanding the immune basis of disease resistance and can inform breeding.

**150.** (C) Marek's Disease
In poultry genetics, the $B_21$ haplotype of the MHC B-complex is well-known for conferring strong resistance against Marek's Disease, a highly contagious viral cancer. Chickens with this haplotype are much less likely to develop tumors after infection. This discovery has had a significant impact on the poultry industry, allowing breeders to select for birds with natural genetic resistance to this disease.

**151.** (D) Peptide-binding groove
The binding of an antigenic peptide to an MHC molecule is mediated by specific anchor residues at each end of the peptide. these residues fit into specialized pockets within the peptide-binding groove of the MHC protein. The interaction between these anchors and the pockets determines which peptides can be held by a specific MHC allele, underlying the specificity of the immune response.

**152.** (A) MHC Class I molecules
Cross-presentation is a specialized immune process that allows certain cells, particularly dendritic cells, to present exogenous antigens on MHC Class I molecules. This is important for activating CD8+ cytotoxic T cells against viruses that do not infect the dendritic cells themselves. It bridges the gap between the exogenous and endogenous antigen presentation pathways, enhancing the versatility of the overall immune response.

**153.** (B) Thymus
T cell maturation in the thymus involves a critical selection process that ensures T cells can recognize MHC molecules but do not react against the body's own proteins. MHC molecules in the thymus present a variety of selF-peptides to developing T cells. Those that bind too strongly or not at all are eliminated, a process that is essential for maintaining immunological selF-tolerance.

**154.** (B) Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)
In swine immunology, specific haplotypes of the SLA system have been found to influence the immune response to the Porcine Reproductive and Respiratory Syndrome Virus. These genetic variations can affect how well a pig responds to infection or vaccination. Understanding these associations is a key part of efforts to improve pig health and resilience to this economically devastating respiratory disease.

**155.** (A) Downregulate MHC Class I expression
Natural Killer cells are a component of the innate immune system that can detect and kill abnormal cells without prior sensitization. One way they identify targets is by monitoring the levels of MHC Class I molecules on the cell surface. Many viruses and cancer cells downregulate MHC Class I to avoid detection by T cells, but this absence triggers the NK cells to attack.

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**156.** (C) Broad specificity
MHC molecules exhibit broad specificity, meaning that each individual allele can bind a wide variety of different peptides as long as they share certain anchor residues. However, a single peptide will only bind to a specific set of MHC alleles. This property allows a limited number of MHC genes to present the diverse array of antigens encountered by the immune system.

**157.** (D) Circular double-stranded DNA
The mitochondrial genome in farm animals, as in other mammals, is a small, circular, double-stranded DNA molecule. Unlike the linear chromosomes found in the cell nucleus, the mtDNA is not associated with histones and is present in multiple copies within each mitochondrion. This unique structural organization reflects the evolutionary origin of mitochondria and is essential for its replication and function.

**158.** (B) 16.5 kb
In most livestock species, the complete mitochondrial genome is approximately 16.5 kilobases in size. This compact genome is highly efficient, with very little non-coding DNA between genes. Despite its small size, it encodes critical components for energy production. The consistency in size across different mammalian species highlights the functional constraints

on the mitochondrial genome throughout its evolutionary history in various animals.

**159.** (B) Maternal inheritance
Mitochondrial DNA is inherited almost exclusively through the maternal line. During fertilization, the egg provides the vast majority of the cytoplasm and organelles to the zygote, while the mitochondria in the sperm are typically degraded. This uniparental inheritance pattern means that all offspring share their mother's mitochondrial genotype, making it a powerful tool for tracing maternal lineages and evolutionary history.

**160.** (A) 13
The mammalian mitochondrial genome universally encodes exactly 13 protein-coding genes. These genes are essential for the assembly and function of the mitochondrial respiratory chain. In addition to these proteins, the mtDNA encodes several other molecules necessary for its own independent translation system. All other proteins required for mitochondrial function are encoded by the nuclear genome and imported into the organelle.

**161.** (A) Oxidative phosphorylation
The 13 proteins encoded by the mitochondrial DNA are all essential subunits of the enzyme complexes involved in oxidative phosphorylation. This process occurs in the inner mitochondrial membrane and is the cell's primary way of generating ATP. Because these proteins are central to energy production, mutations in their genes can have significant effects on the metabolism and health of the animal.

**162.** (C) 22
The mitochondrial genome encodes 22 different transfer RNA molecules. These tRNAs are required for the translation of the 13 protein-coding genes within the mitochondrion itself. The set of 22 tRNAs is sufficient for mitochondrial translation because of the unique way they recognize codons, which is slightly different from the system used for nuclear-encoded mRNAs in the cytoplasm of the cell.

**163.** (C) 10 to 17 times higher
Mitochondrial DNA has a significantly higher mutation rate than nuclear DNA, often estimated to be 10 to 17 times faster. This increased rate of sequence change makes mtDNA a highly sensitive marker for studying recent evolutionary events and fine-scale population structure. The accumulation of these mutations over time allows researchers to distinguish between different maternal lineages and domestic animal breeds.

**164.** (D) Lack of histones and efficient repair
Several factors contribute to the high mutation rate of mitochondrial DNA. Unlike nuclear DNA, mtDNA is not protected by histones and lacks the highly efficient repair mechanisms found in the nucleus. Additionally, mitochondria are a major source of reactive oxygen species, which can directly damage the DNA. These conditions lead to a faster accumulation of genetic variations compared to the nuclear genome.

**165.** (B) Heteroplasmy
Heteroplasmy is a condition where an individual or a single cell contains more than one type of mitochondrial DNA. This can occur due to a new mutation or the inheritance of different mtDNA molecules. Because there are many mitochondria per cell, the ratio of these different types can change during cell division, which may influence the severity of mitochondrial-related traits or diseases.

**166.** (C) Tryptophan
The genetic code used in mammalian mitochondria differs slightly from the universal genetic code. One notable difference is that the codon UGA, which is a stop codon in the nucleus, encodes the amino acid Tryptophan in the mitochondrion. These variations in the code are a unique feature of mitochondrial evolution and require specialized translation machinery within the organelle to produce proteins.

**167.** (D) Heavy (H) and Light (L) strands
The two strands of the circular mitochondrial genome are named the Heavy strand and the Light strand based on their different base compositions. The Heavy strand is rich in Guanine and encodes the majority of the mitochondrial genes, including 12 of the 13 proteins. The Light strand is rich in Cytosine and encodes only one protein-coding gene and a few transfer RNAs.

**168.** (A) Introns
A defining characteristic of mitochondrial genes is that they completely lack introns, which are non-coding sequences found within nuclear genes. This results in a very compact genome

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where genes are often located immediately adjacent to each other. This lack of splicing requirements simplifies the transcription and translation processes within the mitochondrion, reflecting its evolutionary roots and the need for high metabolic efficiency.

**169.** (B) Displacement loop (D-loop)
The displacement loop, or D-loop, is the main non-coding region of the mitochondrial genome. It is highly polymorphic and evolves at a much faster rate than the protein-coding regions. Because of this high level of variation, sequencing the D-loop is a standard method for phylogenetic analysis, allowing researchers to study the genetic diversity, domestication history, and maternal relationships of livestock.

**170.** (B) Origin of replication for the Heavy strand and promoters for transcription
The D-loop is a critical regulatory region of the mitochondrial DNA. It contains the primary origin of replication for the Heavy strand and the promoters that initiate transcription for both strands. Because it controls these essential processes, the D-loop is central to the maintenance and expression of the mitochondrial genome. Its non-coding nature allows it to accumulate mutations without affecting function.

**171.** (A) Maternal lineage and domestication history
Sequencing the mitochondrial D-loop allows researchers to trace the maternal ancestry of livestock populations. Because mtDNA is inherited from the mother, variations in this region can be used to reconstruct maternal lineages and understand the geographical origins and domestication history of different breeds. This information is valuable for managing genetic diversity and for protecting the heritage of traditional livestock animals.

**172.** (C) Originated from independent domestication centers of wild aurochs
Mitochondrial DNA analysis has provided key insights into the domestication of cattle. By sequencing the D-loop, researchers have established that taurine cattle and indicine cattle originated from independent domestication events of different wild aurochs populations in separate regions. This confirms that these two main types of domestic cattle have distinct maternal origins rather than a single common domestication ancestor in the past.

**173.** (D) The high copy number of mtDNA per cell improves recovery chances
Mitochondrial DNA is often preferred for studying ancient DNA from archaeological livestock remains. This is primarily because each cell contains hundreds to thousands of copies of the mitochondrial genome, compared to only two copies of nuclear DNA. This high copy number significantly increases the chances of recovering enough genetic material from degraded samples to allow for successful sequencing and phylogenetic analysis.

**174.** (C) Haplogroup
A haplogroup is a large group of individuals who share a common ancestor as defined by specific mutations in their mitochondrial DNA. These groups are monophyletic, meaning all members descend from a single lineage. In livestock research, identifying the distribution of different haplogroups helps scientists understand how ancient populations moved and how modern breeds were formed through domestication and breeding.

**175.** (A) A, B, C, D, and E
Studies of mitochondrial DNA in domestic sheep have identified five major maternal lineages, which are categorized into haplogroups A, B, C, D, and E. These haplogroups show distinct geographical distributions, reflecting multiple domestication events or early migrations. Haplogroups A and B are the most common worldwide, and their study provides a detailed picture of the genetic history of sheep.

**176.** (D) Maximum Likelihood or Neighbor-Joining
To reconstruct the evolutionary relationships between livestock breeds, researchers align their mitochondrial sequences and use algorithms to build phylogenetic trees. Maximum Likelihood and Neighbor-Joining are two commonly used methods for this purpose. These algorithms use the differences in the DNA sequences to estimate the most likely branching order and divergence times, providing a visual representation of the evolutionary history.

**177.** (A) The divergence time between distinct haplogroups
The molecular clock concept is based on the idea that mutations in certain DNA regions, like the mitochondrial D-loop, accumulate at a relatively steady rate over time. By measuring

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splice junctions. STAR is a key component of many transcriptomic pipelines, providing the necessary foundation for subsequent steps like gene quantification and the analysis of differential gene expression in animals.

**197.** (B) FeatureCounts
After the RNA-Seq reads have been aligned to the reference genome, the next step is to count how many reads map to each gene or feature. FeatureCounts is a commonly used tool for this quantification. It is fast and efficient, providing a table of raw counts that can then be normalized and used for identifying genes that are differentially expressed between samples.

**198.** (C) Sequencing depth and gene length
Normalization is an essential step in RNA-Seq analysis to ensure that differences in read counts between samples are due to actual biological variation rather than technical factors. Common normalization methods adjust for differences in total sequencing depth and for the length of the genes. This allows for the fair comparison of gene expression levels across different samples and between genes.

**199.** (D) Normalized gene expression metrics
FPKM and TPM are two commonly used metrics for reporting normalized gene expression levels in RNA-Seq studies. FPKM adjusts for both the total number of reads in the sample and the length of the gene, while TPM is a similar metric that is increasingly preferred because it is easier to compare directly between samples. Both provide a standardized measure of transcript abundance.

**200.** (A) Differential Gene Expression (DGE) analysis
Differential Gene Expression analysis is the statistical process used to identify genes whose expression levels change significantly between different experimental conditions, such as healthy versus diseased animals. This analysis uses normalized read counts to identify genes that are up-regulated or down-regulated. Identifying these genes is a primary goal of many transcriptomic studies, providing insights into the molecular biology of traits.

**201.** (B) Negative Binomial distribution
Software packages like DESeq2 and edgeR use the negative binomial distribution to model the variation in RNA-Seq read counts. This distribution is more appropriate than the Poisson distribution because it can account for "overdispersion," where the variance in expression levels between biological replicates is greater than the mean. This leads to more accurate and reliable identification of differentially expressed genes.

**202.** (B) $LoG_2$ fold-change versus statistical significance (p-value)
A Volcano Plot is a useful way to visualize the results of a differential gene expression study. It plots the statistical significance of each gene's expression change against the magnitude of that change, often as a $loG_2$ fold-change. This allows researchers to quickly identify the genes that are most significantly and strongly up-regulated or down-regulated, highlighting them for further biological investigation.

**203.** (A) Identify overrepresented biological pathways
After identifying differentially expressed genes, researchers often perform enrichment analyses to understand their biological significance. Gene Ontology and KEGG pathway analyses identify which biological processes or metabolic pathways are overrepresented among the changing genes. This helps to provide a higher-level view of the molecular changes occurring in the animal and identifies the key biological systems affected by the condition.

**204.** (C) A high-quality reference genome is unavailable
De novo transcriptome assembly is a method for reconstructing transcripts directly from RNA-Seq reads without the use of a reference genome. This approach is essential for studying species that do not yet have a high-quality genome sequence. Tools like Trinity are used to assemble the short reads into longer contiguous sequences, providing a foundation for understanding the transcriptome in these organisms.

**205.** (D) An entire community of microorganisms extracted directly from an environment
Metagenomics is a field of study that involves sequencing the genetic material from entire communities of microorganisms directly from environmental or biological samples. Unlike traditional genomics, which focuses on individual cultured species, metagenomics provides a comprehensive view of all the microbes present, including those that cannot be grown in a

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**187.** (A) Distinguish between overlapping transcripts from the sense and antisense strands
Strand-specific RNA-Seq methods allow researchers to determine which of the two DNA strands was used as the template for a given transcript. This is highly advantageous because it helps distinguish between genes that overlap on opposite strands. It also allows for the accurate identification of antisense transcripts, which play important regulatory roles but can be confused with sense transcripts.

**188.** (C) Illumina Sequencing-by-Synthesis
Illumina sequencing-by-synthesis is the most widely used technology for short-read RNA-Seq in livestock research. This platform produces millions of short, high-quality sequences, or reads, which are ideal for quantifying gene expression levels and identifying splice variants. Its high throughput and relatively low cost per base have made it the standard choice for most transcriptomic studies in various animal species.

**189.** (D) Transcriptional profiles and heterogeneity of individual cells
Single-cell RNA-Seq is an advanced technique that allows for the transcriptomic profiling of individual cells. Unlike bulk RNA-Seq, which provides an average measurement across a whole tissue, scRNA-Seq reveals the diversity and heterogeneity within a cell population. This allows researchers to identify rare cell types, study cell differentiation, and understand how individual cells respond differently to environmental stimuli or diseases.

**190.** (C) Adding unique index barcodes to the adapters of each sample
To increase efficiency and reduce costs, multiple RNA-Seq samples can be sequenced together in a single lane. This is achieved by adding unique DNA barcodes, or index sequences, to the adapters of each sample during library preparation. After sequencing, the bioinformatics software uses these barcodes to assign each read back to its original sample, a process known as demultiplexing.

**191.** (B) 150-300 bp
Short-read sequencing platforms like Illumina are most effective when analyzing DNA fragments that are between 150 and 300 base pairs long. Because original RNA transcripts can be much longer, they or the resulting cDNA must be fragmented into smaller pieces. This ensures that the fragments are within the optimal range for the sequencing chemistry, resulting in high-quality data and efficient coverage.

**192.** (A) $> 8$
The quality of the starting RNA is crucial for successful RNA-Seq. The RNA Integrity Number is a score from 1 to 10 that indicates the level of degradation in a sample, with 10 being perfectly intact. High-quality transcriptomic studies typically require an RIN score greater than 8. Using intact RNA ensures that the sequencing library represents the variety of transcripts accurately.

**193.** (C) Quality control (QC) and adapter trimming
The first step in analyzing raw RNA-Seq data is quality control and adapter trimming. This involves using software to check the quality of the reads and to remove any remaining adapter sequences or low-quality bases from the ends. This preprocessing ensures that only high-quality data is used for subsequent steps like alignment, which improves the accuracy of the expression measurements.

**194.** (C) FastQC
FastQC is a popular and easy-to-use software tool that provides a comprehensive report on the quality of raw sequencing data in FASTQ format. It checks various metrics, such as per-base quality scores, GC content, and the presence of overrepresented sequences. This allows researchers to quickly identify any issues with the sequencing run or the library preparation before proceeding with analysis.

**195.** (A) Spliced out introns
In eukaryotes, many genes contain introns that are removed during splicing. When messenger RNA is sequenced, the resulting reads often span across these splice junctions. Therefore, bioinformatics pipelines must use "splice-aware" aligners that can correctly map these reads to the reference genome by accounting for the gaps created by the missing introns. This is essential for accurately quantifying transcript isoforms.

**196.** (D) STAR
STAR is a highly efficient and widely used splice-aware read aligner designed for RNA-Seq data. It is capable of rapidly mapping millions of reads to a reference genome while accurately identifying

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the number of differences between sequences, researchers can estimate when two lineages last shared a common ancestor. This allows for the dating of important evolutionary events, such as the timing of domestication.

**178.** (C) Gray Wolf
Genetic studies using mitochondrial DNA sequencing have definitively shown that the domestic dog is descended from the gray wolf. By comparing the mtDNA of many dog breeds with various wild canids, researchers found that dogs share a close maternal relationship exclusively with wolves. This discovery has helped clarify the evolutionary origin of the dog and the timing of domestication.

**179.** (A) More conserved and useful for deeper interspecies phylogeny
The cytochrome b gene is a protein-coding gene in the mitochondrial genome that is often used for phylogenetic studies. Compared to the hypervariable D-loop, the cytochrome b sequence is more conserved, making it particularly useful for investigating the relationships between different species or more distantly related groups. It provides a more stable evolutionary signal for studying deeper branches in animal phylogeny.

**180.** (D) Fertile Crescent (Near East)
Analysis of mitochondrial DNA has localized the domestication center for taurine cattle to the Fertile Crescent in the Near East. The major taurine haplogroups, such as $T_3$, show a clear origin in this region, followed by a spread into Europe and Africa alongside human migration. This confirms the Near East as a primary source of the cattle lineages that dominate.

**181.** (B) The transcriptome (RNA transcripts)
RNA-Seq is a powerful technology that uses high-throughput sequencing to analyze the transcriptome, which is the complete set of RNA transcripts produced by a cell or tissue. This method allows researchers to identify which genes are active and to measure their levels of expression under different conditions. It provides a detailed snapshot of the molecular activity within an organism.

**182.** (C) Oligo(dT) probes
In eukaryotic cells, mature messenger RNAs are characterized by a poly-A tail at their 3' ends. To isolate these transcripts for RNA-Seq, researchers use magnetic beads coated with oligo-dT probes. The probes bind specifically to the poly-A tails, allowing the mRNA to be separated from the much more abundant ribosomal and transfer RNAs. This step focuses the analysis on protein-coding genes.

**183.** (C) rRNA (approx. 80-90%)
The vast majority of the total RNA in a mammalian cell, approximately 80% to 90%, is composed of ribosomal RNA. rRNA forms the core of the ribosomes, which are the machines that synthesize proteins. Because rRNA is so dominant, it must often be removed or depleted before sequencing to allow for the effective detection of less abundant transcripts like messenger RNA.

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**184.** (A) Ribosomal RNA depletion (Ribo-Zero)
For studies that need to include non-polyadenylated transcripts, such as certain non-coding RNAs or bacterial RNAs, researchers use ribosomal RNA depletion instead of poly-A selection. This process, often called Ribo-Zero, uses specific probes to remove the ribosomal RNA from the sample. This leaves all other types of RNA behind, providing a more comprehensive view of the total transcriptomic landscape.

**185.** (B) Reverse transcription into complementary DNA (cDNA)
Because most high-throughput sequencing platforms are designed for DNA, the isolated RNA must be converted into a more stable form before analysis. This is achieved through reverse transcription, where the enzyme reverse transcriptase uses the RNA as a template to synthesize a complementary DNA molecule. The resulting cDNA represents the transcript population and can be easily processed into a sequencing library.

**186.** (B) Adapter ligation
Adapter ligation is a critical step in preparing a library for sequencing. It involves attaching short, synthetic DNA sequences to the ends of the cDNA fragments. These adapters provide the necessary binding sites for the sequencing primers and for attaching the fragments to the flow cell of the sequencer. They are essential for making the DNA compatible with the specific platform.

laboratory. This allows for a much broader understanding of diversity.

**206.** (C) The taxonomic composition and relative abundance of bacterial and archaeal populations
16S rRNA amplicon sequencing is a widely used method in metagenomics to profile the taxonomic composition of bacterial and archaeal communities. By targeting a specific region of the 16S ribosomal RNA gene, researchers can identify the different types of microbes present and their relative abundance in a sample. This approach is highly efficient for studying the microbiome across various environments.

**207.** (A) Highly conserved regions for universal primer binding and hypervariable regions for species identification
The 16S rRNA gene is a powerful tool for microbial phylogeny because it contains both highly conserved and hypervariable regions. The conserved regions allow for the design of universal primers that can amplify the gene from almost all bacteria. The hypervariable regions, on the other hand, provide the sequence diversity needed to distinguish between different species and to reconstruct relationships.

**208.** (D) Operational Taxonomic Units (OTUs)
In the analysis of 16S rRNA sequences, reads that are highly similar are often clustered together into Operational Taxonomic Units. These OTUs are typically defined based on a 97% sequence identity threshold, which is a traditional proxy for distinguishing different microbial species. Clustering into OTUs helps to simplify complex metagenomic data, allowing for the comparison of microbial communities across different samples.

**209.** (A) Exact sequence-level resolution distinguishing single nucleotide differences
Amplicon Sequence Variants are an alternative to OTUs that provide higher resolution in metagenomic studies. Instead of clustering sequences based on a similarity threshold, ASV analysis identifies unique sequences with single-nucleotide resolution. This allows researchers to distinguish between closely related microbial strains and provides a more precise and reproducible way to characterize the composition of microbial communities in various samples.

**210.** (C) Sequencing all DNA in the sample, capturing functional genes and non-bacterial taxa
Whole-Genome Shotgun metagenomics involves sequencing all the DNA fragments in a sample, rather than focusing on a single marker gene. This approach provides a comprehensive view of the entire genetic potential of the microbial community, including functional genes and the genomes of viruses and fungi. It allows for the profiling of metabolic pathways and the discovery of new microbial species.

**211.** (A) Alpha diversity
Alpha diversity is a metric used to describe the microbial diversity within a single sample. It takes into account both the number of different species present, known as richness, and their relative abundance, known as evenness. Common measures of alpha diversity include the Shannon and Simpson indices. These metrics provide a summary of the complexity of a local microbial community.

**212.** (D) The difference in microbial community composition between different samples or environments
Beta diversity is a measure of the differences in microbial community composition between different samples or environments. It allows researchers to quantify how similar or different two communities are based on the types and abundances of the microbes they contain. This is often visualized using ordination plots, which show how samples cluster together based on their overall microbial profiles.

**213.** (B) Antibiotic resistance genes and metabolic pathways
A significant advantage of shotgun metagenomics is that it provides information about the functional potential of a microbial community. In addition to identifying the species present, researchers can profile the genes involved in metabolic pathways, antibiotic resistance, and other biological processes. This allows for a deeper understanding of what the microbes are actually doing and how they interact with their environment.

**214.** (C) Metagenome-Assembled Genomes (MAGs)
Metagenome-Assembled Genomes are reconstructed by grouping DNA fragments from shotgun metagenomic data that belong to the same microorganism. This process, known

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these proteins was discovered to be predictable, leading to its use as a powerful and flexible tool for genome editing.

**254.** (A) 1 base pair of DNA
The DNA-binding domain of a TALE protein is made up of a series of highly similar repeats, each about 34 amino acids long. Crucially, each individual repeat is responsible for recognizing exactly one base pair in the target DNA sequence. This one-to-one relationship between the repeats and the DNA bases makes the design of TALENs much more straightforward and predictable.

**255.** (B) Repeat-Variable Diresidue (RVD)
The specificity of each TALE repeat for a particular DNA base is determined by two specific amino acids at positions 12 and 13, known as the Repeat-Variable Diresidue. By varying these two residues, researchers can create TALE proteins that bind to any desired sequence of DNA. The discovery of this RVD code allowed for the development of easily programmable TALENs.

**256.** (A) Adenine (A)
According to the TALE RVD code, a repeat that contains the amino acids Asn-Ile at positions 12 and 13 will specifically recognize and bind to the nucleotide Adenine. By assembling a sequence of TALE repeats with the appropriate RVDs, researchers can create a DNA-binding domain that precisely matches any target sequence in the genome, allowing for targeted genome editing.

**257.** (B) Cytosine (C)
In the modular code used by TALE proteins, the Repeat-Variable Diresidue consisting of His-Asp is specific for the nucleotide Cytosine. This predictable relationship between the RVD and the target base is what makes TALENs so versatile. Any sequence of DNA can be targeted simply by stringing together the correct series of repeats, each with the RVD corresponding to the nucleotide.

**258.** (C) FokI
Similar to ZFNs, TALENs are created by fusing an engineered DNA-binding domain to the cleavage domain of the FokI restriction enzyme. Because FokI must dimerize to cut DNA, two TALENs are required to bind to opposite strands of the target site. This arrangement ensures that a double-strand break is only introduced at the specific location where both TALENs are correctly bound.

**259.** (A) Completely free of context dependence, making design predictable
A major advantage of TALENs over ZFNs is that their DNA-binding domains are much more modular and lack the context-dependence issues seen with zinc fingers. This means that a TALE repeat will recognize its target base regardless of the sequences recognized by the surrounding repeats. This predictability makes the design of effective TALENs much faster and more reliable for livestock research.

**260.** (B) Thymine (T)
Natural TALE proteins have a structural requirement that their target DNA sequence must begin with a Thymine nucleotide. This is due to a specific interaction between the first repeat-like domain of the TALE protein and the DNA. When designing TALENs, researchers must take this requirement into account by selecting target sites that start with a T for efficient binding.

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**261.** (B) The highly repetitive nature of the DNA sequence encoding the TALE repeats, prone to recombination
Constructing the DNA sequences that encode TALEN arrays can be challenging because the TALE repeats are highly similar to each other. This high level of repetition makes the DNA prone to recombination and difficult to assemble using standard methods. Specialized techniques, such as Golden Gate cloning or solid-phase assembly, are used to overcome these issues and build TALEN genes.

**262.** (C) POLLED (PC) locus on chromosome 1
In a landmark study, researchers used TALENs to introduce a genetic variation into dairy cattle that produces the polled, or hornless, phenotype. They targeted the POLLED locus on chromosome 1, successfully introgressing a naturally occurring mutation from beef cattle into a Holstein lineage. This demonstrated the power of genome editing to improve animal welfare by eliminating physical dehorning procedures.

**263.** (D) The optimal distance for FokI dimerization and cleavage
When designing a pair of TALENs, the size of the spacer region between the two binding sites is a critical factor for successful editing. The

strategies to manipulate the rumen microbiome to improve the productivity of livestock.

**224.** (D) 3-Nitrooxypropanol (3-NOP)
3-Nitrooxypropanol, also known as 3-NOP, is a chemical compound that has been shown to significantly reduce methane emissions from cattle. It works by specifically inhibiting the enzyme methyl-coenzyme M reductase, which is required for the final step of methanogenesis in rumen archaea. Research into the effects of 3-NOP on the rumen microbiome is ongoing to ensure its safety and effectiveness.

**225.** (A) Bromoform
Supplementing the diets of ruminants with certain types of seaweed, particularly Asparagopsis taxiformis, can lead to a dramatic reduction in enteric methane production. This effect is primarily due to the presence of bromoform, a bioactive compound that interferes with the methanogenesis pathway in rumen archaea. This natural approach is being investigated as a potential way to lower the environmental impact.

**226.** (C) Subacute Ruminal Acidosis (SARA)
When ruminants are switched from a high-fiber forage diet to a high-grain concentrate diet, the rumen microbiome undergoes a rapid and dramatic change. This shift can lead to the overproduction of lactic acid and a significant drop in rumen pH, a condition known as Subacute Ruminal Acidosis. SARA is a major health concern in the dairy and beef industries, affecting animal productivity.

**227.** (A) Propionate
The production of propionate in the rumen is an alternative pathway for hydrogen utilization that competes with methanogenesis. Increasing the proportion of propionate relative to other volatile fatty acids can act as a hydrogen sink, thereby reducing the amount of hydrogen available for methane production. Dietary strategies that favor propionate-producing bacteria are one way to improve animal efficiency and reduce methane.

**228.** (D) Carbohydrate-Active enZymes (CAZymes)
Shotgun metagenomics has allowed for the discovery of many new enzymes from the rumen that are highly effective at breaking down plant cell walls. These enzymes, collectively known as Carbohydrate-Active enZymes, include cellulases and hemicellulases that could have important applications in the biofuels and food industries. Identifying these novel biocatalysts is a key benefit of studying the complex microbial world.

**229.** (B) Double-Strand Break (DSB)
All modern technologies for programmable genome editing, such as CRISPR-Cas9, ZFNs, and TALENs, function by creating a precise double-strand break in the target DNA. This targeted break initiates the cell's natural DNA repair mechanisms, which can be exploited to introduce specific genetic changes. The ability to direct these breaks to exact genomic locations is the foundation of precise engineering.

**230.** (C) Non-Homologous End Joining (NHEJ)
After a double-strand break is induced in the DNA, the cell most commonly uses the Non-Homologous End Joining pathway to repair the damage. NHEJ is an efficient but error-prone process that often results in the loss or gain of a few nucleotides at the repair site. These small mutations can disrupt the gene's function, making NHEJ a powerful tool for knockouts.

**231.** (D) Indels (insertions/deletions)
The repair of double-strand breaks by the NHEJ pathway frequently introduces small insertions or deletions, collectively known as indels. These mutations often cause frameshifts in the coding sequence of a gene, leading to the production of a non-functional protein or the premature termination of translation. Indels are the primary tool used by researchers to create gene knockouts in livestock.

**232.** (A) Homology-Directed Repair (HDR)
To introduce a specific DNA sequence or a new gene at a targeted site, researchers rely on the Homology-Directed Repair pathway. This process requires a donor DNA template that shares homology with the sequences flanking the double-strand break. By providing this template, the cell can be induced to incorporate the new sequence during the repair process, allowing for precise "knock-in" genetic modifications.

**233.** (B) Late S and $G_2$
Homology-Directed Repair is a highly precise but less common repair mechanism that is primarily active during the late S and $G_2$ phases of the cell cycle. This timing ensures that a sister chromatid is available to serve as a template

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for repair. Because of this restriction, HDR is generally less efficient than NHEJ for genome editing, particularly in early-stage embryos.

**234.** (B) Targeted region of the host genomic DNA
In the context of genome editing, the protospacer is the specific sequence of genomic DNA that is targeted for cleavage by an engineered nuclease. In the CRISPR system, this sequence is complementary to the spacer sequence in the guide RNA. The accurate recognition of the protospacer by the nuclease is what ensures the high specificity and precision of the genetic modification.

**235.** (A) Premature Stop Codon (PTC)
A common strategy for creating a gene knockout in livestock is to use genome editing to induce a frameshift mutation. This is typically achieved by creating a small indel that shifts the reading frame, leading to the occurrence of a premature stop codon. The presence of a PTC prevents the synthesis of a full-length protein, effectively silencing the gene's function.

**236.** (C) OfF-target effect
OfF-target effects occur when a genome-editing nuclease cleaves the DNA at a location other than the intended target site. This happens when there are other regions in the genome with sequences that are similar enough to the target for the nuclease to bind and cut. Minimizing these unintended mutations is a major challenge in ensuring the safety and precision of engineering.

**237.** (D) Zygote's pronucleus
One common method for generating genome-edited livestock is the microinjection of editing reagents directly into the pronucleus of a zygote. This approach allows the nuclease to act at the earliest stage of development, ideally leading to a modification that is present in every cell of the resulting animal. However, this technique can sometimes result in mosaicism if editing occurs later.

**238.** (C) Fully clonal and uniformly edited, avoiding mosaicism
Somatic Cell Nuclear Transfer is frequently combined with genome editing to produce animals with precise and uniform genetic changes. In this process, somatic cells are edited and screened in culture, and a successfully modified cell is then used as a nuclear donor to create a clone. This approach ensures that all the cells in the resulting animal carry the same intended edit.

**239.** (A) One-cell embryo stage
Mosaicism occurs when an animal has different genotypes in different cells, which can happen if a genome-editing nuclease remains active after the one-cell embryo stage. If some cells are edited while others are not, or if different edits occur in different cells, the resulting founder animal will be a mosaic. This can complicate the characterization of the phenotype and inheritance.

**240.** (C) Significantly lower
In mammalian embryos, the efficiency of Homology-Directed Repair is generally much lower than that of Non-Homologous End Joining. This makes it more difficult to achieve precise gene knock-ins compared to simple gene knockouts. Researchers are developing new methods to improve the rate of HDR, such as using small molecules to inhibit NHEJ or by delivering the donor template effectively.

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**241.** (B) 14 to 40 bp
Meganucleases are natural DNA-cleaving enzymes that recognize very long target sequences, typically between 14 and 40 base pairs. This long recognition site makes them highly specific, as such a sequence is unlikely to occur more than once in a genome. Meganucleases were the first tools used for targeted genome editing, but their complex structure makes it difficult to redesign them.

**242.** (C) Complex structural recognition constraints
A major challenge with using meganucleases for genome editing is the difficulty in engineering them to recognize new target sequences. Because the DNA-binding and cleavage functions are tightly integrated into a single protein domain, changing the specificity often requires complex and labor-intensive protein engineering. This limitation led to the development of more modular and easily programmable tools like ZFNs and TALENs.

**243.** (A) FokI
Zinc Finger Nucleases were the first widely used artificial nucleases for genome editing. They are created by fusing a series of zinc finger DNA-binding domains to the non-specific cleavage domain of the FokI restriction enzyme. The zinc

fingers provide the target specificity, while the FokI domain provides the ability to cut the DNA, allowing for site-specific double-strand breaks.

**244.** (B) 3 bp
The DNA-binding domain of a ZFN consists of multiple zinc finger motifs, each of which is designed to recognize and bind to a specific three-base-pair sequence of DNA. By stringing together several of these motifs, researchers can create a protein that recognizes a longer, unique target sequence. This modular design is what allows ZFNs to be programmed to target genes.

**245.** (B) A pair (Left and Right ZFNs)
The FokI cleavage domain used in ZFNs only becomes active when two of these domains come together to form a dimer. Therefore, for genome editing to occur, two separate ZFNs must be designed to bind to adjacent sites on opposite strands of the DNA. This requirement for dimerization increases the specificity of the system, as it requires two independent binding events.

**246.** (B) 6
To target a specific 18-base-pair sequence on a single DNA strand, a ZFN would typically require an array of six individual zinc finger motifs, as each finger recognizes three base pairs. A pair of such ZFNs, binding to 18 base pairs on each side of the cleavage site, would provide a very high level of specificity for genetic modifications.

**247.** (D) 5' overhangs (sticky ends)
When two ZFNs bind to their target sites and the FokI domains dimerize, they introduce a double-strand break in the DNA. This cleavage typically results in fragments with 5' overhangs, also known as sticky ends. The specific structure of these ends can influence the way the cell's repair machinery processes the break, impacting the efficiency and types of mutations.

**248.** (A) The binding specificity of one zinc finger was influenced by its neighboring fingers
A significant technical issue with early ZFNs was context dependence, where the binding of one zinc finger was affected by the sequences recognized by its neighbors. This made it difficult to predict how well a newly designed ZFN would bind to its intended target. Overcoming this problem required extensive testing and the development of better libraries of zinc finger motifs.

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**249.** (B) Zinc ($Zn^{2+}$)
The structural integrity of a zinc finger motif is maintained by the coordination of a single zinc ion. The ion is typically held in place by two cysteine and two histidine residues, which are characteristic of the Cys2-His2 type of zinc finger. This metal ion is essential for the protein to fold into the correct shape needed to recognize specific DNA bases.

**250.** (B) Prevent hyperacute rejection in xenotransplantation
ZFN technology was used to create the first knockout pigs lacking the $GGTA_1$ gene, which encodes an enzyme responsible for producing a specific sugar molecule called alpha-gal. This sugar is found on the surface of pig cells and causes hyperacute rejection when pig organs are transplanted into humans. This breakthrough was a major step forward in the field of xenotransplantation research.

**251.** (A) Formation of active FokI homodimers at solitary binding sites
Of F-target cleavage by ZFNs can occur if a single ZFN binds to a site and then forms a homodimer with another copy of itself. This can lead to unintended double-strand breaks at genomic locations that contain only one of the two intended binding sites. Reducing the ability of FokI domains to form homodimers while maintaining their ability to form heterodimers is key.

**252.** (C) Two distinct, uniquely engineered FokI variants bind adjacent to each other
Researchers have developed "obligate heterodimer" versions of the FokI cleavage domain to improve the specificity of ZFNs and TALENs. These engineered domains are designed so that they can only form a functional dimer when two different variants are present. This prevents the formation of homodimers, thereby reducing ofF-target cleavage at sites that are only partially similar to the target.

**253.** (D) Plant pathogenic bacteria of the genus Xanthomonas
Transcription Activator-Like Effector Nucleases were developed based on proteins found in Xanthomonas, a genus of bacteria that causes diseases in plants. These bacteria secrete TALE proteins into plant cells to manipulate host gene expression. The modular DNA-binding domain of

as binning, allows researchers to obtain near-complete genomes of uncultured microbes. MAGs have revolutionized our understanding of microbial diversity by providing genetic information for many species that have never been isolated or grown in a laboratory setting.

**215.** (D) "Dark matter"
Microbial "dark matter" refers to the vast majority of microorganisms that have never been cultured or characterized in a laboratory. these microbes are only known through their DNA sequences found in metagenomic studies. Understanding this hidden diversity is a major goal of modern microbiology, as these organisms may play critical roles in their ecosystems and could be sources of biotechnological tools.

**216.** (A) Whether the microbial diversity in the sample has been exhaustively sequenced
A rarefaction curve is a graphical representation used in metagenomics to determine if the sequencing depth is sufficient to capture the full diversity of a sample. It plots the number of observed species against the number of reads sequenced. When the curve levels off, it suggests that further sequencing will not significantly increase the number of species found, indicating adequate coverage.

**217.** (B) Rumen
The rumen is the largest compartment of the ruminant stomach and serves as a massive fermentation vat. It houses a complex community of bacteria, archaea, fungi, and protozoa that break down complex plant polysaccharides, such as cellulose and hemicellulose. This microbial activity is essential for the host animal's nutrition, as it converts fibrous feed into energy-rich compounds for the animal.

**218.** (B) Bacteroidetes and Firmicutes
The rumen microbiome is consistently dominated by two main bacterial phyla: Bacteroidetes and Firmicutes. These groups are responsible for the majority of the fermentation and degradation of dietary components in the rumen. The balance between these phyla can change depending on the animal's diet and environment, but they remain the core members of the microbial community in ruminants.

**219.** (A) Volatile Fatty Acids (VFAs) like acetate, propionate, and butyrate
Rumen microbes ferment complex carbohydrates into volatile fatty acids, which are the primary energy source for ruminants. The most important VFAs produced are acetate, propionate, and butyrate. These compounds are absorbed through the rumen wall and enter the animal's bloodstream, providing up to 70% of its total energy requirement. This symbiotic relationship is fundamental to the biology of livestock.

**220.** (C) Archaea
Methanogenesis is a specialized metabolic process that occurs in the rumen, resulting in the production of methane gas. This process is carried out exclusively by a group of microorganisms called methanogens, which belong to the domain Archaea. Although they make up only a small fraction of the total rumen microbiome, their activity has significant implications for animal efficiency and the environment.

**221.** (D) Hydrogen gas ($H_2$)
Rumen methanogens produce methane by using hydrogen gas to reduce carbon dioxide. This process is an important part of rumen fermentation, as it helps to maintain a low partial pressure of hydrogen, which is necessary for the efficient degradation of plant material by other microbes. However, the resulting methane is a potent greenhouse gas that is released into the atmosphere.

**222.** (B) Constitute a loss of 2% to 12% of the animal's gross energy intake
The production of methane in the rumen represents a significant loss of energy for the host animal. It is estimated that between 2% and 12% of the gross energy intake of a ruminant is lost in the form of methane. Reducing this loss is a major goal for livestock producers, as it could improve feed efficiency while also decreasing the environmental footprint.

**223.** (B) Feed Conversion Ratio (FCR) / Residual Feed Intake (RFI)
Metagenomic studies of the rumen have found that the composition of the microbial community is strongly associated with the feed efficiency of the host animal. Specific microbial profiles are correlated with traits like Residual Feed Intake and Feed Conversion Ratio. By understanding these relationships, researchers hope to develop

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spacer must be of the correct length to allow the two FokI domains to dimerize and cleave the DNA efficiently. If the spacer is too short or too long, the cleavage efficiency will be significantly reduced.

**264.** (A) TALENs require engineering a completely new, large protein for every new DNA target
Although TALENs are highly specific and effective, they have a disadvantage compared to CRISPR-Cas9 in terms of ease of use. For every new genomic target, a completely new and relatively large protein must be engineered and assembled. This makes TALENs less suitable for large-scale experiments or screens where hundreds of different genes need to be targeted for editing.

**265.** (B) Adaptive immunity against invading bacteriophages and plasmids
The CRISPR-Cas system was originally discovered in bacteria and archaea, where it serves as a form of adaptive immunity. It allows these microorganisms to recognize and destroy the DNA of invading viruses or plasmids that they have encountered before. This natural defense mechanism has been repurposed by scientists into a versatile and powerful tool for precise genome editing in livestock.

**266.** (B) Single-guide RNA (sgRNA)
The revolutionary CRISPR-Cas9 system uses a single-guide RNA to direct the Cas9 nuclease to its target DNA site. The specificity of the system is determined by the sequence of the sgRNA, which can be easily designed to match any genomic location. This RNA-guided approach is much simpler and more flexible than the protein-guided methods used by ZFNs and TALENs.

**267.** (A) tracrRNA
The single-guide RNA used in genome editing is a synthetic molecule that combines two parts of the natural CRISPR system: the crRNA and the tracrRNA. The crRNA contains the sequence that matches the target DNA, while the tracrRNA provides the structural framework that allows the sgRNA to bind to the Cas9 protein. Together, they form a complex that guides cleavage.

**268.** (C) Protospacer Adjacent Motif (PAM)
For the Cas9 enzyme to bind and cut its target DNA, the sequence must be immediately adjacent to a short, specific motif known as the Protospacer Adjacent Motif. The PAM is recognized directly by the Cas9 protein and is essential for the initiation of the DNA-RNA pairing process. Without the correct PAM, Cas9 will not cleave the target DNA.

**269.** (B) 5'-NGG-3'
The most commonly used version of the Cas9 enzyme, derived from Streptococcus pyogenes, requires a specific PAM sequence consisting of the nucleotides NGG, where N is any base. This requirement means that researchers must select target sites in the genome that are immediately followed by an NGG motif. This sequence occurs frequently, providing many potential targets for genome editing.

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**270.** (C) RuvC and HNH
Cas9 is a large protein that functions as a DNA endonuclease, using two distinct domains to cut the two strands of the DNA. The HNH domain cleaves the DNA strand that is complementary to the guide RNA, while the RuvC domain cleaves the non-complementary strand. Together, these two domains introduce a precise double-strand break at the targeted location within the genome.

**271.** (B) Blunt ends approximately 3 bp upstream of the PAM
The cleavage performed by the wild-type Cas9 enzyme typically results in a blunt-ended double-strand break. This break occurs at a specific position, generally three base pairs upstream of the PAM sequence. The nature of this blunt end distinguishes Cas9 from other nucleases like FokI, and it has implications for how the cell's DNA repair machinery will respond to the damage.

**272.** (D) Targeted gene silencing (CRISPRi) or activation (CRISPRa) without cutting DNA
A catalytically inactive, or "dead," version of the Cas9 protein can be created by mutating its nuclease domains. While dCas9 can still be guided to a specific DNA site by an sgRNA, it can no longer cut the DNA. This allows dCas9 to be used as a targeted delivery vehicle for other proteins, such as those that activate or silence genes.

**273.** (B) 20 nt
The specificity of the CRISPR-Cas9 system is provided by a short "spacer" sequence within the guide RNA, which is typically 20 nucleotides

antigen, Agrobacterium infection, integration into genome

2001

**9.** According to the Jerne immune network theory, an anti-idiotypic vaccine mimics the original antigen by utilizing which of the following?
(A) The specific Fc region of the primary antibody isotype
(B) The constant domain of a hyperimmune serum molecule
(C) The peptide-binding cleft of the T-cell receptor
(D) The internal image of the Ab2 variable region

2024

**10.** When designing synthetic peptide vaccines, coupling the peptide to a carrier protein like KLH is primarily done to overcome which limitation?
(A) Lack of T-helper cell epitopes in the peptide
(B) Intrinsic high toxicity of the naked peptide sequence
(C) Rapid renal clearance of the peptide from circulation
(D) Presence of uncontrolled peptide conformational changes

2003

**11.** What is the fundamental requirement for a successful DIVA (Differentiating Infected from Vaccinated Animals) strategy?
(A) Induction of a stronger response than natural infection
(B) Absence of a specific antigen found in wild-type strains
(C) Detection of specific cross-reactive antibodies from species
(D) Administration exclusively via the primary mucosal route

2011

**12.** Reverse vaccinology differs from traditional vaccinology primarily by beginning the discovery process at which level?
(A) Standard in vitro cultivation of the viral pathogen
(B) In silico bioinformatic analysis of the pathogen genome
(C) Initial in vivo challenge using established animal models
(D) Biochemical purification of specific surface proteins

2022

**13.** Which technique is most central to the field of structural vaccinology for rational antigen design?
(A) X-ray crystallography
(B) Flow cytometry
(C) Polymerase chain reaction
(D) Enzyme-linked immunosorbent assay

2024

**14.** In systems vaccinology, transcriptomic profiling is primarily utilized to achieve which objective?
(A) Purification of the final formulated vaccine product
(B) Measuring the exact halF-life of serum antigen levels
(C) Prediction of host immune responses and vaccine efficacy
(D) Sterilization of the production bioreactor before use

2015

**15.** Adversomics involves the immunogenetic profiling of a population to understand which of the following phenomena?
(A) The divergence of viral strains over time
(B) Codon usage optimization for plasmid vectors
(C) Degradation kinetics of formulated mRNA vaccines
(D) Genetic basis of severe adverse reactions to vaccines

2004

**16.** Match the adjuvant in List I with its primary mechanism of action in List II:
List I: (Adjuvant)
P. Aluminum salts
Q. Freund's Complete Adjuvant
R. CpG oligodeoxynucleotides
S. ISCOMs
List II: (Mechanism)
1. TLR9 agonism
2. Depot formation and NLRP3 inflammasome activation
3. Delivery of antigen to the cytosol for MHC-I cross-presentation
4. Mycobacterial components driving intense Th1 and Th17 responses
(A) P-2, Q-4, R-1, S-3
(B) P-4, Q-2, R-3, S-1
(C) P-2, Q-4, R-3, S-1
(D) P-3, Q-1, R-4, S-2

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several subgroups of ALV. Deleting this gene using genome editing prevents the virus from entering the cells, providing a genetic method for protecting poultry from this common infection.

**284.** (C) PRNP
Prion diseases, such as Bovine Spongiform Encephalopathy, are caused by the misfolding of the normal prion protein encoded by the PRNP gene. Researchers have shown that cattle in which the PRNP gene has been completely knocked out using genome editing are fully resistant to prion infection. These animals appear healthy, suggesting the protein is not essential for normal growth.

**285.** (D) Transgenesis via genome editing
A more complex strategy for creating disease-resistant livestock involves using genome editing to "knock in" functional genetic elements, such as those that produce antiviral RNAs or neutralizing antibodies. Because this approach introduces new genetic material into the genome at a specific site, it is considered a form of transgenesis that is guided and made more precise by technology.

**286.** (C) Rosa26
Researchers have generated pigs that are resistant to the Classical Swine Fever Virus by inserting a gene that produces anti-CSFV small hairpin RNAs into the Rosa26 locus. This locus is a "safe harbor," meaning it can support the expression of new genes without affecting the animal's normal health. The shRNAs work by specifically degrading the viral RNA, blocking infection.

**287.** (B) Pigs
Pigs have been successfully genome-edited to lack the CD163 receptor, which has been shown in controlled challenge trials to provide complete resistance to the PRRS virus. These animals do not become infected and do not show any of the typical symptoms of the disease. This is a significant breakthrough for animal health and for the productivity of the pig industry.

**288.** (D) Do not contain any foreign DNA and mimic naturally occurring mutations
One of the potential benefits of using targeted gene knockouts to create disease-resistant animals is that the resulting modifications can be very similar to naturally occurring mutations. Unlike traditional transgenesis, these animals may not contain any foreign or bacterial DNA. This could lead to a more favorable regulatory environment and better public acceptance of genome-edited livestock for food production.

**289.** (A) Celtic POLLED allele (Pc)
The development of horns in cattle is a major concern for animal welfare and farmer safety. A naturally occurring genetic variant, known as the Celtic POLLED allele, is known to prevent horn growth in certain beef breeds. Researchers have used genome editing to introgress this dominant allele into dairy cattle, providing a way to produce hornless animals without physical dehorning.

**290.** (C) Avoidance of multi-decade backcrossing
The use of genome editing to introduce the POLLED trait into dairy cattle is much faster than traditional crossbreeding. In just one generation, researchers can produce a purebred dairy animal with the hornless phenotype, a process that would otherwise take decades of backcrossing. This demonstrates how technology can rapidly improve livestock by incorporating beneficial traits from other breeds without losing productivity.

**291.** (A) Prolactin receptor (PRLR) gene
Heat stress is a major factor limiting the productivity of cattle in warm climates. Some breeds have a naturally occurring "slick" coat phenotype, which is associated with better heat tolerance. Researchers have identified that mutations in the Prolactin Receptor gene are responsible for this trait. By using genome editing to introduce these mutations into other breeds, they can improve resilience.

**292.** (D) Tropical and subtropical climates
Cattle with the slick coat mutation have a shorter and sparser hair coat, which helps them lose heat more efficiently through sweating and convection. Introducing this trait into high-yielding dairy breeds could allow them to maintain high levels of production in tropical and subtropical regions where heat stress is a significant problem. This is a clear example of using genetics for adaptation.

**293.** (B) Milk allergy in human infants
Beta-lactoglobulin is a major protein in the milk of cows and other ruminants, but it is not found in human milk. For this reason, it is one of the

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long. This sequence is designed to be perfectly complementary to the target DNA site. The ability of the Cas9-sgRNA complex to recognize and bind to this specific 20-base-pair sequence is what allows for the precise targeting of locations.

**274.** (C) Deaminase enzyme
Base editors are a newer type of CRISPR-based tool that can directly change one DNA base into another without creating a double-strand break. These tools consist of a modified Cas9 protein fused to a deaminase enzyme, which can chemically convert one nucleotide to another, such as Cytosine to Uracil. This allows for highly precise single-base changes in the genome.

**275.** (A) Reverse transcriptase and a pegRNA
Prime editing is a versatile and highly precise genome-editing technology that combines a modified Cas9 nuclease with a reverse transcriptase enzyme. It uses a specialized guide RNA, called a pegRNA, which not only directs the complex to the target site but also contains the new genetic information to be inserted. This system allows for a wide range of edits.

**276.** (D) Delivering multiple different sgRNAs with a single Cas9 protein
One of the most powerful features of the CRISPR-Cas9 system is its ability to perform multiplex editing, where several different genes are modified simultaneously. This is achieved by simply delivering multiple different guide RNAs, each targeting a different genomic site, along with a single Cas9 protein. This approach is much more efficient than using ZFNs or TALENs for multiple changes.

**277.** (B) CD163
The Porcine Reproductive and Respiratory Syndrome Virus is a major threat to the pig industry worldwide. To infect its host, the virus must bind to a specific receptor on the surface of porcine macrophages. This primary receptor has been identified as CD163. Understanding the role of this protein in viral entry has provided a key target for developing resistant pigs.

**278.** (C) Scavenger receptor cysteine-rich (SRCR) domain 5
Researchers have used CRISPR-Cas9 to create pigs that are resistant to the PRRS virus by modifying the CD163 receptor. Specifically, they targeted and deleted the scavenger receptor cysteine-rich domain 5 of the protein, which is the part used by the virus to enter the cell. This precise modification blocks the virus from infecting the animal while leaving the gene intact.

**279.** (D) Retain the normal biological functions of the CD163 protein
Deleting only the SRCR domain 5 of the CD163 protein is a strategic approach for creating PRRS-resistant pigs. This modification is sufficient to prevent viral entry but is small enough that the protein can still perform its normal biological functions in the pig's immune system. This targeted deletion avoids potential negative health effects that might arise from knocking out the gene.

**280.** (A) RELA (p65 subunit of NF-κB)
African Swine Fever is a devastating viral disease of pigs for which there is no effective vaccine. Researchers are studying the genetic basis of the natural tolerance found in warthogs, focusing on genes like RELA. By using genome editing to introduce warthog-like variations into domestic pigs, they hope to create animals that are better able to survive the ASFV infection.

**281.** (B) NRAMP1 ($SLC11A_1$) or SP110
Efforts to create cattle that are resistant to bovine tuberculosis have focused on genes involved in the immune response of macrophages, such as $SLC11A_1$ and SP110. By using genome editing to increase the expression of these genes or to introduce beneficial mutations, researchers have successfully produced cattle that show improved resistance to the bacteria that cause this serious disease.

**282.** (B) Enteric (intestinal) coronavirus diseases
Coronaviruses like TGEV and PEDV cause severe intestinal disease in pigs, leading to high mortality in piglets. These viruses use the ANPEP protein as a receptor to enter the cells of the gut. By using CRISPR-Cas9 to knock out or modify the ANPEP gene, researchers are working to develop pigs that are naturally resistant to these enteric infections.

**283.** (A) tvb gene
Resistance to Avian Leukosis Virus in chickens can be achieved by targeting the host genes that encode the receptors used by the virus. Specifically, the tvb gene encodes a receptor for

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# CHAPTER 10
# Vaccine Technology

2021

**1.** Which of the following describes the primary advantage of passive immunization in veterinary medicine?
(A) It induces robust long-term mucosal memory
(B) It provides immediate but temporary humoral protection
(C) It activates antigen-specific cytotoxic T lymphocytes
(D) It initiates a rapid secondary immune response upon exposure

2009

**2.** The attenuation of traditional live viral vaccines is most commonly achieved through which of the following methodologies?
(A) Chemical inactivation using formaldehyde
(B) Admixture with aluminum-based adjuvants
(C) Extraction of surface glycoproteins
(D) Repeated serial passage in non-natural host cell cultures

2004

**3.** During the kinetic profile of a primary humoral immune response to a bacterial pathogen, which immunoglobulin isotype typically peaks first?
(A) Immunoglobulin M
(B) Immunoglobulin A
(C) Immunoglobulin E
(D) Immunoglobulin G

2013

**4.** Consider the following statements regarding recombinant subunit vaccines using prokaryotic expression systems:
I. They often lack appropriate post-translational modifications.
II. They pose a high risk of reverting to virulence.
III. They frequently require powerful adjuvants to be immunogenic.
Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I

2019

**5.** Which of the following best explains why Poxviruses are highly effective as vaccine vectors?
(A) They can accommodate large insertions of foreign DNA
(B) They possess a very small genome that prevents recombination
(C) They replicate entirely in the nucleus of the host cell
(D) They integrate permanently into the host chromosomal DNA

2024

**6.** A rationally attenuated live vaccine created via targeted gene deletion is most likely to eliminate which specific element?
(A) A major structural protein of the viral capsid
(B) The primary origin for viral genomic replication
(C) The entire host-derived lipid envelope structure
(D) A non-essential virulence or immunomodulatory gene

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2002

**7.** In DNA vaccine technology, which regulatory element is typically utilized to drive high-level expression of the antigen in mammalian cells?
(A) The $T_7$ bacteriophage-specific promoter
(B) Cytomegalovirus (CMV) early promoter
(C) The prokaryotic LacZ operon promoter
(D) The insect-specific polyhedrin promoter

2015

**8.** Which sequence correctly represents the general order of events in producing an Agrobacterium-mediated plant-based vaccine?
(A) Gene insertion into T-DNA, Agrobacterium infection of plant tissue, integration into plant genome, expression of antigen
(B) Agrobacterium infection, gene insertion into T-DNA, expression of antigen, integration into genome
(C) Integration into plant genome, Agrobacterium infection, gene insertion into T-DNA, expression of antigen
(D) Gene insertion into T-DNA, expression of

2024
**26.** Which type of immunity is established when a newborn calf receives protective antibodies through the ingestion of maternal colostrum?
(A) Natural passive immunity
(B) Natural active immunity
(C) Artificial active immunity
(D) Artificial passive immunity

2014
**27.** The process of chemical inactivation in vaccine preparation frequently utilizes beta-propiolactone. What is its primary target?
(A) Viral lipid envelopes
(B) Nucleic acids
(C) Carbohydrate capsules
(D) Peptidoglycan cell walls

2001
**28.** Which component of the immune system is primarily responsible for clearing an established intracellular viral infection following vaccination?
(A) Complement classical pathway
(B) Cytotoxic CD8+ T lymphocytes
(C) Neutrophil extracellular traps
(D) Eosinophilic degranulation

2023
**29.** When utilizing eukaryotic expression systems like baculovirus/insect cells for subunit vaccines, what is the primary benefit over Escherichia coli systems?
(A) Capability for complex post-translational modifications
(B) Elimination of all fermentation equipment needs
(C) Faster doubling times and lower media costs
(D) Complete absence of endotoxin production

2025
**30.** Consider the following statements regarding Salmonella vectors:
I. They are naturally adapted to mucosal surfaces.
II. They are typically administered via intramuscular injection.
III. They can deliver DNA vaccines directly to host cells.
IV. They are engineered by deleting essential metabolic genes like aroA.
Which of the above statement(s) is/are INCORRECT?
(A) I and II
(B) II and III
(C) I, III, and IV
(D) II

2019
**31.** Rational attenuation of a bacterial pathogen for vaccine use can be achieved by the deletion of genes responsible for synthesizing which of the following?
(A) Ribosomal RNA
(B) DNA polymerase
(C) Phospholipid bilayer components
(D) Essential metabolic intermediates (e.g., purines)

2025
**32.** Plasmids used in DNA vaccines require a bacterial origin of replication (ori) for which specific purpose?
(A) To initiate transcription in the vaccinated animal
(B) To allow the plasmid to integrate into the host animal's genome
(C) To enable amplification and production of the plasmid in E. coli prior to formulation
(D) To induce apoptosis in antigen-presenting cells

2003
**33.** Chloroplast engineering in plant expression systems is highly advantageous for vaccine production due to which feature?
(A) Rapid secretion of the antigen into the surrounding soil
(B) High levels of antigen accumulation without gene silencing
(C) Absolute prevention of all post-translational modifications
(D) Dependence on viral promoters exclusively

2022
**34.** In the context of anti-idiotypic vaccines, the "Ab1" antibody refers to which of the following?
(A) The antibody produced against the anti-idiotype
(B) The monoclonal antibody used as an adjuvant
(C) The non-specific immunoglobulin in the host serum
(D) The primary antibody raised against the original target antigen

2023
**35.** The identification of B-cell epitopes for synthetic peptide vaccines relies primarily on finding regions of the antigen that exhibit which property?
(A) High hydrophilicity and surface exposure
(B) High hydrophobicity and internal burial
(C) Transmembrane spanning sequences

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2010

**17.** Which of the following cytokines is most commonly incorporated into vaccine formulations to drive a polarized Th1 immune response?
(A) Interleukin-4
(B) Interleukin-12
(C) Interleukin-10
(D) Transforming Growth Factor-beta

2019

**18.** Polymeric PLGA microspheres are advantageous as particulate vaccine delivery systems because they exhibit which characteristic?
(A) They function as replication-competent viral vectors
(B) They integrate into the host cellular membrane system
(C) They effectively block the endosomal escape of antigens
(D) They provide controlled release and are biodegradable

2015

**19.** Virus-like particles (VLPs) differ from fully attenuated live viruses in which critical aspect?
(A) VLPs lack genetic material and cannot replicate
(B) VLPs fail to display conformational viral epitopes
(C) VLPs cannot be processed by antigen-presenting cells
(D) VLPs require metallic adjuvants to induce a response

2022

**20.** In large-scale vaccine production, what is the primary advantage of using suspension cell cultures over adherent cell cultures?
(A) They require significant amounts of serum-heavy media
(B) They are entirely resistant to all bacterial contaminants
(C) They scale easily in bioreactors without microcarriers
(D) They exclusively produce high titers of mucosal antibody

2008

**21.** During the lyophilization process of a vaccine, what is the specific role of a cryoprotectant such as sucrose or trehalose?
(A) Prevention of ice formation and protection of structure
(B) Action as a broad-spectrum antimicrobial preservative
(C) Enhancing the recognition of the antigen by dendritic cells
(D) Adjusting the pH of the final reconstituted product

2002

**22.** Target animal safety testing for a new veterinary vaccine must evaluate which of the following?
(A) Protective efficacy against heterologous challenge only
(B) Safety in three distinct non-target mammalian species
(C) Efficacy of the specific companion diagnostic assay
(D) Overdose administration and repeated administration

2005

**23.** Identify the INCORRECT statement regarding the pre-clinical phases of vaccine development.
(A) Immunogenicity trials measure capacity to induce responses
(B) Field efficacy trials use gnotobiotic animal models
(C) Dose determination studies optimize the antigen payload
(D) Challenge studies evaluate protection against infection

2007

**24.** Which of the following is a post-production quality control test primarily used to confirm the absence of extraneous agents in a viral vaccine batch?
(A) In vivo potency assay
(B) Bradford protein assay
(C) Sterility and purity testing
(D) Adjuvant emulsification test

2002

**25.** Read the following statements regarding toxoid vaccines and choose the correct option:
**Assertion (A):** Toxoid vaccines require the addition of an adjuvant to induce a robust and long-lasting immune response.
**Reason (R):** Toxoids are highly immunogenic multivalent structures that naturally stimulate strong toll-like receptor signaling.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

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primary causes of milk allergy in human infants who are fed cow's milk. Removing this protein could lead to the production of hypoallergenic milk that is safer for consumption by sensitive individuals.

**294.** (C) Beta-lactoglobulin (BLG)
Scientists have successfully used genome editing to produce hypoallergenic milk by knocking out the gene for beta-lactoglobulin in cattle. The resulting milk lacks this specific allergenic protein while retaining other important components. This demonstrates the potential for genetic engineering to improve the nutritional and safety profile of livestock products for human consumption, addressing specific health needs in the global population.

**295.** (D) Omega-3 fatty acids
To improve the health benefits of pork, researchers have used genome editing to introduce the fat-1 gene from the nematode C. elegans into pigs. This gene allows the animals to convert less healthy omega-6 fatty acids into beneficial omega-3 fatty acids. This results in meat that has a more favorable lipid profile, potentially reducing the risk of heart disease for consumers.

**296.** (A) UCP1 gene
Piglets often struggle with cold stress because they lack functional brown adipose tissue, which is used by other mammals to generate heat. Researchers have addressed this by using CRISPR-Cas9 to knock a functional UCP1 gene into the pig genome. The resulting pigs have functional brown fat and are much better at maintaining their body temperature, improving survival in cold environments.

**297.** (B) Significant muscle hypertrophy and increased meat yield
The knockout of the myostatin gene has been one of the most common applications of genome editing in livestock. In all species tested, including cattle, pigs, and sheep, the absence of functional myostatin leads to a significant increase in muscle mass and a reduction in body fat. This results in higher meat yields and improved carcass quality, providing a clear economic benefit.

**298.** (B) Phytase
The Enviropig was a genetically engineered pig designed to produce the enzyme phytase in its saliva. This allowed the pig to digest the phosphorus in its feed more efficiently, significantly reducing the amount of phosphorus excreted in its manure. This was a proactive approach to addressing the environmental problem of water pollution caused by high-phosphorus runoff from intensive pig farms.

**299.** (A) $KISS_1$ or its receptor
To address the welfare issues associated with surgical castration, researchers have explored the use of genome editing to create pigs that do not reach puberty. By knocking out genes like $KISS_1$, they can prevent the production of the hormones that lead to sexual maturation and the development of boar taint. This could eliminate the need for castration while ensuring high-quality meat.

**300.** (C) $GGTA_1$
A key challenge for xenotransplantation is the presence of the alpha-gal epitope on pig cells, which is recognized by the human immune system and causes organ rejection. Genome editing has been used to knock out the $GGTA_1$ gene, which is responsible for synthesizing this sugar molecule. This modification is a critical part of making pig organs more compatible for transplantation into human patients.

**********

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(D) Catalytic active site residues

2024

**36.** Which diagnostic companion test is most commonly paired with a targeted gene-deletion DIVA vaccine?
(A) Western blot for the deleted gene product
(B) PCR targeting the host genome
(C) Competitive ELISA detecting antibodies against the deleted antigen
(D) Agglutination test for total immunoglobulin

2023

**37.** Which statement accurately describes a major limitation of reverse vaccinology?
(A) Inability to identify non-protein antigens like polysaccharides
(B) Requires laborious cultivation of dangerous pathogens in vitro
(C) It cannot predict the presence of open reading frames in genomes
(D) It only functions for viral pathogens and not bacterial species

2006

**38.** Structural vaccinology was instrumental in the design of stabilized pre-fusion antigens for which viral component type?
(A) Matrix proteins
(B) Non-structural proteases
(C) Nucleocapsid proteins
(D) Viral envelope fusion glycoproteins

2018

**39.** Vaccinomics leverages multi-omics data primarily to address which challenge in vaccine development?
(A) The high cost of glass vials
(B) The individual and population-level variability in vaccine responses
(C) The lack of bioreactor capacity
(D) The geographical distribution of wild-type pathogens

2018

**40.** Determine the correct sequence for selecting an appropriate adjuvant during vaccine formulation.
(A) Identify the desired immune response profile, evaluate compatibility with the antigen, conduct safety and toxicity testing, assess long-term stability
(B) Conduct safety testing, assess long-term stability, identify immune profile, evaluate compatibility
(C) Evaluate compatibility, identify immune profile, assess long-term stability, conduct safety testing
(D) Assess long-term stability, conduct safety testing, evaluate compatibility, identify immune profile

2019

**41.** Saponin-based adjuvants, such as Quil A, are known for their ability to strongly induce which specific type of immune response?
(A) Purely Th2 humoral responses
(B) Regulatory T-cell suppression
(C) Mast cell degranulation
(D) Cytotoxic T lymphocyte (CTL) responses and Th1 immunity

2021

**42.** Chemokines utilized as biological vaccine enhancers primarily function by promoting which mechanism?
(A) Recruitment of antigen-presenting cells to the site of immunization
(B) Direct neutralization of the viral pathogen
(C) Degradation of foreign DNA
(D) Cross-linking of B-cell receptors

2014

**43.** Liposomal encapsulation protects vaccine antigens primarily from which in vivo process prior to cellular uptake?
(A) Opsonization by complement
(B) Proteolytic and enzymatic degradation
(C) Renal filtration
(D) Exocytosis

2024

**44.** Nanogels utilized in targeted mucosal vaccine delivery offer which distinct biochemical advantage?
(A) They are rigid, impermeable crystalline structures
(B) They possess mucoadhesive properties and respond to environmental stimuli (e.g., pH)
(C) They entirely bypass the lymphatic system
(D) They act as powerful systemic immunosuppressants

2017

**45.** In bioreactor operations for vaccine production, maintaining the dissolved oxygen (DO) concentration is critical. How is this typically regulated?
(A) By adjusting the sparging rate of oxygen/air and impeller agitation speed
(B) By altering the pH of the culture medium
(C) By cooling the reactor to 4°C
(D) By adding excessive amounts of antifoam

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agents

2010

**46.** Match the vaccine formulation excipient in List I with its function in List II:

List I: (Excipient)

P. Thimerosal

Q. Polysorbate 80

R. Sucrose

S. Aluminum hydroxide

List II: (Function)

1. Surfactant preventing protein aggregation
2. Antimicrobial preservative
3. Adjuvant enhancing immunogenicity
4. Lyoprotectant stabilizing structure during freeze-drying

(A) P-2, Q-1, R-4, S-3

(B) P-4, Q-2, R-1, S-3

(C) P-2, Q-4, R-1, S-3

(D) P-1, Q-2, R-3, S-4

2007

**47.** National regulatory authorities evaluating a veterinary vaccine's batch purity primarily look for the absence of which contaminant?

(A) The vaccine antigen itself

(B) The approved adjuvant

(C) Lyophilization stabilizers

(D) Mycoplasma and adventitious viruses

2008

**48.** During dose determination in pre-clinical trials, the "minimum immunizing dose" is defined as what?

(A) The lowest dose that causes adverse effects

(B) The dose that induces sterile immunity in 100% of animals

(C) The lowest antigen payload required to induce a statistically significant protective immune response

(D) The absolute lowest measurable quantity of protein

2004

**49.** In vitro potency testing of a killed bacterial vaccine batch is progressively replacing in vivo challenge models. What is a standard in vitro metric?

(A) Lethal dose 50 (LD50) in mice

(B) Quantification of specific antigen mass using ELISA

(C) Observation of clinical signs in target species

(D) Measurement of fever reduction

2010

**50.** Read the following statements and choose the correct option:

**Assertion (A):** Killed viral vaccines generally require multiple booster doses to maintain protective immunity.

**Reason (R):** Inactivated antigens typically do not replicate in the host and therefore provide a shorter duration of antigenic stimulation.

(A) Both A and R are true and R is the correct explanation of A.

(B) Both A and R are true but R is not the correct explanation of A.

(C) A is true but R is false.

(D) A is false but R is true.

2014

**51.** The secondary immune response is characterized by which of the following parameters compared to the primary response?

(A) Longer lag phase and lower antibody titer

(B) Shorter lag phase, faster rise, and higher plateau of IgG

(C) Predominance of IgM with minimal somatic hypermutation

(D) Complete absence of memory T cell involvement

NODIA

2004

**52.** What is a primary disadvantage of using traditional live-attenuated vaccines in veterinary populations?

(A) Failure to elicit a cellular immune response

(B) Requirements for high doses of chemical adjuvants

(C) Risk of disease in immunocompromised animals

(D) Prohibitively high costs of recombinant manufacture

2004

**53.** Secretory IgA (sIgA) plays a crucial role in mucosal immunity by executing which primary function?

(A) Complement-mediated lysis of epithelial cells

(B) Pathogen exclusion through mucosal neutralization

(C) Intracellular digestion of phagocytosed bacteria

(D) Activation of the classical complement cascade

2005

**54.** Consider the following statements regarding eukaryotic expression systems (e.g., yeast) for vaccine production:

I. They can perform glycosylation.
II. They are entirely incapable of folding complex proteins.
III. They secrete endotoxins that must be removed.
IV. They are generally more expensive to scale up than E. coli.

Which of the above statement(s) is/are correct?

(A) I and II
(B) I and IV
(C) II and III
(D) III and IV

2011

**55.** Adenovirus vectors are engineered for vaccine delivery primarily by deleting the $E_1$ region. What is the consequence of this deletion?

(A) It renders the vector replication-defective in normal host cells
(B) It causes the vector to replicate uncontrollably
(C) It prevents the vector from entering target cells
(D) It increases the virulence of the adenovirus

2011

**56.** Which of the following describes a "temperature-sensitive" rationally attenuated mutant?

(A) Strain replicating well at core body temperatures but inactive at mucosal temperatures
(B) Mutant replicating at low temperatures but not at core body or febrile temperatures
(C) A strain that requires boiling to activate its immunogenic epitopes
(D) A strain that induces hyperthermia in the host

2011

**57.** The cellular uptake mechanism of naked plasmid DNA vaccines is significantly enhanced by which physical method?

(A) Lyophilization
(B) In vivo electroporation
(C) Homogenization
(D) Sonication prior to injection

2009

**58.** Edible vaccines produced in transgenic plants face which major regulatory and physiological hurdle?

(A) Standardizing the exact antigen dose per consumed serving
(B) Preventing the plants from performing photosynthesis
(C) Total lack of mucosal immunogenicity
(D) Instant degradation of the DNA by sunlight

2022

**59.** The Jerne immune network theory suggests that the immune system is a selF-regulating network of antibodies. What term describes the unique antigenic determinants on the variable region of an antibody?

(A) Isotypes
(B) Allotypes
(C) Idiotypes
(D) Epitopes

2015

**60.** Spatial conformation of synthetic peptide vaccines can be stabilized to mimic native structures by introducing which chemical modification?

(A) Disulfide bridges
(B) Cleavage by restriction endonucleases
(C) Poly-A tails
(D) Ubiquitination

2015

**61.** In the context of DIVA strategies for Foot-and-Mouth Disease (FMD), the diagnostic assay typically detects antibodies against which proteins to identify naturally infected animals?

(A) Structural capsid proteins
(B) Non-structural proteins (NSPs) such as 3ABC
(C) Viral envelope lipids
(D) Host acute phase proteins

2023

**62.** Which database search tool is indispensable in reverse vaccinology for predicting transmembrane domains and signal peptides?

(A) BLAST
(B) PRED-TMBB and SignalP
(C) Clustal Omega
(D) MEGA

2011

**63.** Consider the following statements regarding structural vaccinology:

I. It requires the isolation of the whole pathogen in cell culture.
II. It utilizes cryo-electron microscopy to resolve protein structures.
III. It aims to lock flexible viral proteins into their most immunogenic conformation.
IV. It only applies to bacterial polysaccharide antigens.

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pathogen

2002

**138.** Which parameter is a critical focus of structural vaccinology when designing stabilized trimeric glycoprotein antigens?
(A) Introducing destabilizing mutations to loosen the structure
(B) Removing all glycosylation sites completely
(C) Chopping the protein into 10-amino-acid fragments
(D) Introducing engineered disulfide bonds and proline substitutions to lock the pre-fusion conformation

2008

**139.** Vaccinomics utilizes systems biology. Which algorithm type is frequently used in systems vaccinology to predict T-cell epitopes from genomic data?
(A) Artificial neural networks for MHC binding affinity prediction (e.g., NetMHCpan)
(B) Monte Carlo simulations of viral spread
(C) Fluid dynamics modeling of blood flow
(D) Linear programming for cost reduction

2021

**140.** Consider the following statements regarding adversomics:
I. Genetic variation in HLA/MHC molecules can explain disparate adverse reactions.
II. Adversomics completely ignores the host genetic background.
III. It aims to develop personalized or population-specific safety guidelines.
IV. Polymorphisms in cytokine receptor genes are relevant targets of study.
Which of the above statement(s) is/are correct?
(A) I, II, and III
(B) I, III, and IV
(C) II, III, and IV
(D) I and IV

2023

**141.** The adjuvant MF59, often used to boost responses to influenza vaccines, is primarily composed of an emulsion containing which naturally occurring oil?
(A) Mineral oil
(B) Peanut oil
(C) Linseed oil
(D) Squalene

2024

**142.** If a vaccine formulator wishes to enhance the mucosal immunity (IgA) of an intranasally delivered vaccine, which cytokine might rationally be included as an immunomodulator?
(A) Transforming Growth Factor-beta (TGF-beta)
(B) Interleukin-2 (IL-2)
(C) Tumor Necrosis Factor-alpha (TNF-alpha)
(D) Interferon-alpha

2016

**143.** Which of the following is a primary characteristic of ISCOMs that distinguishes them from simple liposomes?
(A) ISCOMs are composed purely of water
(B) ISCOMs possess an open, cage-like structure formed by saponin, lipid, and antigen
(C) ISCOMs are strictly solid metallic nanoparticles
(D) ISCOMs dissolve instantly upon contact with serum

2018

**144.** In nanotechnology-based vaccinology, Virus-Like Particles (VLPs) assemble spontaneously. What is the fundamental requirement for this selF-assembly?
(A) The presence of the viral RNA genome
(B) The expression of one or more viral structural proteins capable of oligomerization
(C) The presence of a lipid bilayer in all cases
(D) The addition of chemical cross-linkers

2014

**145.** Determine the correct sequence of phases in the downstream processing of a viral vaccine produced in cell culture.
(A) Cell lysis/harvesting, clarification (filtration/centrifugation), purification (chromatography), concentration and formulation
(B) Purification, clarification, concentration, cell lysis
(C) Clarification, concentration, purification, cell lysis
(D) Concentration, purification, cell lysis, clarification

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2024

**146.** Lyophilization relies on three distinct steps. What is the final step, designed to remove tightly bound residual moisture?
(A) Freezing
(B) Primary drying
(C) Secondary drying (desorption)
(D) Annealing

2015

**74.** In post-production testing, an in vivo safety test requires administering multiple times the normal field dose to the target species. What is the standard multiple typically required by regulatory guidelines?
(A) 0.5x the recommended dose
(B) 2x or 10x the recommended dose
(C) 100x the recommended dose
(D) 1000x the recommended dose

2017

**75.** Read the following statements and choose the correct option:
**Assertion (A):** DNA vaccines successfully induce both humoral and cell-mediated immunity without the need for live infectious agents.
**Reason (R):** The plasmid DNA enters host cells and drives the endogenous synthesis of antigen, which is subsequently processed via the MHC Class I and II pathways.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2001

**76.** The concept of "herd immunity" generated by widespread immunization protects which specific sub-population?
(A) Unimmunized or vulnerable individuals in the community
(B) Only those individuals with hyperactive immune systems
(C) Exclusively the previously infected individuals
(D) Only the vaccinated adult males

2012

**77.** Which process is critical for producing an effective bacterin (killed bacterial vaccine) while preserving surface antigenic structures?
(A) Boiling the culture for 60 minutes
(B) Autoclaving the bacterial pellet at 121°C
(C) Extensive proteolysis using trypsin
(D) Treating the culture with formalin under controlled time and temperature

2019

**78.** During the host immune response to an intracellular parasite like Theileria, which T-cell subset is predominantly responsible for target cell lysis?
(A) CD4+ Th2 cells
(B) T regulatory cells
(C) Gamma-delta T cells
(D) CD8+ Cytotoxic T cells

2012

**79.** Which prokaryotic organism is the most commonly utilized host for recombinant protein expression due to its rapid growth and well-characterized genetics?
(A) Bacillus subtilis
(B) Escherichia coli
(C) Staphylococcus aureus
(D) Pseudomonas aeruginosa

2015

**80.** Match the vector type in List I with its descriptive characteristic in List II:
List I: (Vector Type)
P. Fowlpox virus
Q. Attenuated Salmonella
R. Modified Vaccinia Ankara (MVA)
S. Recombinant Adenovirus
List II: (Characteristic)
1. Orally administered vector effective for mucosal targeting
2. Avian vector capable of restricted replication in mammalian cells
3. Highly attenuated orthopoxvirus that fails to replicate in most mammalian cells
4. Non-enveloped vector often hampered by pre-existing vector immunity
(A) P-2, Q-1, R-3, S-4
(B) P-3, Q-2, R-1, S-4
(C) P-1, Q-4, R-2, S-3
(D) P-4, Q-3, R-1, S-2

2003

**81.** A classically attenuated virus may revert to virulence in the field. How does targeted gene deletion prevent this reversion?
(A) Absence of virulence sequences, making recombination improbable
(B) Deleting genes for the envelope, rendering the virus naked
(C) Increasing the mutation rate of the viral polymerase
(D) Linking the virulence gene to a lethal promoter

2016

**82.** What is a primary limitation of RNA vaccine technologies compared to DNA vaccines?
(A) RNA vaccines integrate into the host genome, causing insertional mutagenesis
(B) RNA vaccines cannot be translated into

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Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) I and IV
(D) III and IV

2010
**64.** Which specific "-omics" technology measures the entire complement of proteins produced by an organism to aid in antigen discovery?
(A) Genomics
(B) Transcriptomics
(C) Proteomics
(D) Metabolomics

2023
**65.** The major histocompatibility complex (MHC) genotype of an animal is highly relevant in adversomics. In domestic species, what is the term for the MHC equivalent in dogs?
(A) BoLA
(B) SLA
(C) DLA
(D) EqLA

2014
**66.** Toll-like receptor (TLR) agonists are modern adjuvants. Which TLR is specifically activated by single-stranded RNA (ssRNA) incorporated into a vaccine?
(A) $TLR_4$
(B) $TLR_5$
(C) $TLR_{7/8}$
(D) $TLR_9$

2008
**67.** Which immunomodulator would be least relevant for enhancing an anti-parasitic veterinary vaccine designed to drive a Th2/IgE response?
(A) Interleukin-4
(B) Interleukin-12
(C) Interferon-gamma
(D) Interleukin-13

2024
**68.** Determine the correct order of manufacturing steps for creating a PLGA microsphere vaccine delivery system using the double-emulsion solvent evaporation method.
(A) Primary emulsification ($W_1/O$), secondary emulsification ($W_1/O/W_2$), solvent extraction/evaporation, particle collection/ washing
(B) Secondary emulsification, primary emulsification, particle collection, solvent extraction
(C) Solvent extraction, primary emulsification, secondary emulsification, particle collection
(D) Particle collection, solvent extraction, secondary emulsification, primary emulsification

2008
**69.** Targeting mucosal delivery methods via nanotechnology aims primarily to induce high titers of which antibody at the portal of entry?
(A) Systemic IgG
(B) Secretory IgA
(C) Monomeric IgM
(D) IgD

2021
**70.** In downstream processing of large-scale viral vaccines, which technique is predominantly used for the highly specific purification of a recombinant antigen?
(A) Depth filtration
(B) Affinity chromatography
(C) Lyophilization
(D) Bead milling

2016
**71.** Preservatives are added to multi-dose vaccine vials to prevent which occurrence?
(A) Thermal degradation of proteins
(B) Microbial contamination after the vial is punctured
(C) Aggregation of the adjuvant
(D) Oxidation of the active ingredients

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2006
**72.** Which international organization is primarily responsible for establishing standard diagnostic and vaccine guidelines for terrestrial and aquatic animal diseases?
(A) World Health Organization (WHO)
(B) Food and Agriculture Organization (FAO)
(C) World Organisation for Animal Health (WOAH)
(D) Centers for Disease Control and Prevention (CDC)

2011
**73.** During a clinical field efficacy trial of a veterinary vaccine, which epidemiological parameter measures the reduction in disease incidence among vaccinated animals compared to controls?
(A) Basic reproduction number (R0)
(B) Vaccine efficacy (VE)
(C) Case fatality rate
(D) Point prevalence

granulomas

2014

**92.** The addition of Interleukin-15 (IL-15) to a viral vaccine formulation primarily aims to enhance the development and survival of which immune cells?
(A) Neutrophils
(B) Eosinophils
(C) Basophils
(D) Long-lived memory CD8+ T cells

2006

**93.** ISCOMs (Immune Stimulating Complexes) form cage-like structures. Which three components are fundamentally required to form an ISCOM?
(A) Antigen, cholesterol/phospholipid, and saponin (Quil A)
(B) Antigen, aluminum hydroxide, and lipid A
(C) Antigen, PLGA, and water
(D) Antigen, squalene, and Tween 80

2010

**94.** Nanotechnology applications using dendrimers in vaccinology are characterized by which structural feature?
(A) Linear, unbranched polymer chains
(B) Highly branched, tree-like, symmetrically spherical macromolecules
(C) Flat, two-dimensional graphene sheets
(D) Unstructured, amorphous hydrogels

2015

**95.** When scaling up a bioreactor for vaccine production, the mass transfer of which specific gas is usually the most limiting engineering parameter?
(A) Oxygen
(B) Carbon dioxide
(C) Nitrogen
(D) Methane

2013

**96.** What is the primary reason for adding a buffer (such as phosphate or histidine) to a vaccine formulation?
(A) Increasing viscosity for a depot effect
(B) Action as an internal color expiration indicator
(C) Maintaining optimal pH for antigen stability
(D) Killing adventitious bacterial contaminants

2024

**97.** In the context of veterinary vaccines, what does the "withdrawal period" refer to?
(A) The time required before an animal develops immunity
(B) The mandated time between vaccine administration and slaughter for human consumption
(C) The minimum interval between booster doses
(D) The shelf life of the lyophilized product

2017

**98.** In a clinical trial assessing a novel avian influenza vaccine, target animal safety must be evaluated in which species?
(A) Specific-pathogen-free (SPF) mice
(B) Guinea pigs
(C) The specific avian species for which the vaccine is intended (e.g., chickens)
(D) Non-human primates

2018

**99.** The Limulus amebocyte lysate (LAL) assay is a post-production quality control test used to detect the presence of which substance?
(A) Live viral particles
(B) Bacterial endotoxins (lipopolysaccharides)
(C) Residual formaldehyde
(D) Mycoplasma DNA

2019

**100.** Read the following statements and choose the correct option:
**Assertion (A):** Recombinant protein subunit vaccines are generally safer than live-attenuated vaccines for immunocompromised animals.
**Reason (R):** Subunit vaccines consist only of purified antigenic components and totally lack the capacity to replicate or cause disease.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

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2019

**101.** The immunological memory generated by vaccination primarily resides in which cellular compartments?
(A) Memory B cells and Memory T cells
(B) Long-lived macrophages and neutrophils
(C) Erythrocytes and platelets
(D) Natural killer cells and mast cells

2010

**102.** Which specific methodology converts a potent bacterial exotoxin into a safe toxoid for vaccination?

(A) Digestion with broad-spectrum proteases to create single amino acids
(B) Treatment with heat or formalin to eliminate toxicity while maintaining immunogenicity
(C) Passing the toxin through a 0.22-micron filter
(D) Centrifugation at high speeds to separate the active sub-unit

2023
**103.** Humoral immunity against extracellular bacterial pathogens relies heavily on antibody-dependent mechanisms. Which of the following is NOT one of these primary mechanisms?
(A) Perforin-mediated lysis of the bacteria
(B) Neutralization of toxins
(C) Opsonization to enhance phagocytosis
(D) Activation of the complement cascade

2004
**104.** When selecting a promoter for a eukaryotic expression system (e.g., Chinese Hamster Ovary cells) to produce vaccine antigens, an inducible promoter is often used. Why?
(A) To ensure the antigen immediately degrades upon secretion
(B) To permanently halt cell division
(C) To prevent the antigen from being expressed until the cell culture reaches optimal density
(D) To prevent the need for any subsequent downstream purification

2014
**105.** Poxvirus vectors replicate in the cytoplasm. Why is this a distinct safety advantage for their use as vaccine vectors?
(A) It prevents the vector from producing any proteins
(B) It eliminates the risk of integration into the host's nuclear genome
(C) It ensures the virus cannot be detected by the immune system
(D) It allows the virus to survive indefinitely in the environment

2014
**106.** Match the genetically engineered vaccine approach in List I with its description in List II:
List I: (Approach)
P. Gene deletion mutant
Q. Chimeric virus vaccine
R. Codon deoptimization
S. DNA vaccine
List II: (Description)
1. Utilizing non-preferred synonymous codons to reduce translational efficiency and attenuate the virus
2. Removing a specific virulence gene to create a live-attenuated strain
3. Inserting an antigen gene into a plasmid driven by a mammalian promoter
4. Replacing the structural genes of an attenuated backbone with those of a target pathogen
(A) P-2, Q-4, R-1, S-3
(B) P-4, Q-2, R-1, S-3
(C) P-2, Q-1, R-4, S-3
(D) P-1, Q-4, R-2, S-3

2005
**107.** RNA vaccines typically employ which lipid-based delivery vehicle to ensure cellular uptake and protect the mRNA from degradation?
(A) PLGA microspheres
(B) Lipid nanoparticles (LNPs)
(C) Solid lipid implants
(D) Cholesterol-free liposomes

2002
**108.** Which component in chloroplast engineering prevents the transgene from spreading to wild relatives via pollen?
(A) Chloroplasts are entirely devoid of DNA
(B) Chloroplast DNA is strictly maternally inherited in most plant species, excluding it from pollen
(C) The viral promoter kills the pollen cells
(D) The expressed protein destroys the stamen

2021
**109.** What describes the major theoretical limitation of anti-idiotypic vaccines in large-scale field application?
(A) They cause severe DNA integration
(B) They are highly restricted by individual MHC haplotype variations and may induce tolerance
(C) They are purely composed of lipid A
(D) They require the presence of live wild-type virus

2006
**110.** Consider the following statements regarding the coupling of synthetic peptides to carrier proteins:
I. Tetanus toxoid and Keyhole Limpet Hemocyanin (KLH) are common carrier proteins.
II. Glutaraldehyde or carbodiimides are frequently used as chemical cross-linkers.
III. Coupling diminishes the T-helper response

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to the peptide.
IV. The carrier protein provides necessary T-cell epitopes.

Which of the above statement(s) is/are correct?
(A) I, II, and IV
(B) I and III
(C) II, III, and IV
(D) I, II, and III

2025
**111.** The gE-deleted Bovine Herpesvirus-1 (BoHV-1) vaccine is a classic example of a DIVA vaccine. A naturally infected animal will test:
(A) Negative for gE antibodies
(B) Negative for all BoHV-1 antibodies
(C) Positive for gE DNA in the serum
(D) Positive for gE antibodies

2017
**112.** In reverse vaccinology, "pan-genomic screening" implies analyzing what data set?
(A) Only the core genes of a single reference strain
(B) The genome of the host animal
(C) The transcriptomics of the cell culture media
(D) The genomes of multiple strains of a pathogen to identify conserved, universally protective surface antigens

2002
**113.** Structural vaccinology techniques revealed that the F protein of Respiratory Syncytial Virus (RSV) must be stabilized in which conformation to elicit the highest titer of neutralizing antibodies?
(A) Pre-fusion state
(B) Post-fusion state
(C) Denatured state
(D) Cleaved trimer state

2024
**114.** In Vaccinomics, high-throughput flow cytometry (CyTOF) or mass cytometry is used to achieve which level of analysis?
(A) Sequencing the host genome
(B) Identifying the structural coordinates of an antigen
(C) Measuring the viscosity of the adjuvant
(D) Deep profiling of thousands of individual immune cells simultaneously based on multiple surface markers

2014
**115.** An adverse vaccine reaction characterized by immediate hypersensitivity (anaphylaxis) is mediated primarily by which immunoglobulin isotype cross-linking on mast cells?
(A) IgA
(B) IgG
(C) IgM
(D) IgE

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2006
**116.** Which of the following accurately describes the mechanism of action of saponin derivatives (e.g., QS-21) as adjuvants?
(A) They intercalate into cell membranes, creating pores that facilitate cytosolic delivery of antigens for CD8+ T cell activation
(B) They act purely as physical depots without cellular interaction
(C) They competitively bind to the T-cell receptor blocking activation
(D) They neutralize pro-inflammatory cytokines

2016
**117.** The use of Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) as a biological vaccine enhancer primarily targets the maturation and activation of which critical cell type?
(A) Erythrocytes
(B) Dendritic cells
(C) Plasma cells
(D) Platelets

2016
**118.** When designing liposomal encapsulation for vaccines, altering the lipid composition to include cationic lipids generally serves what purpose?
(A) To enhance electrostatic interaction with negatively charged DNA or RNA molecules
(B) To repel the liposome from cell membranes
(C) To increase the freezing temperature of the formulation
(D) To induce immediate breakdown in the bloodstream

2022
**119.** Targeted mucosal delivery utilizing mucoadhesive polymers like chitosan benefits from which inherent property of chitosan?
(A) It tightly opens tight junctions between epithelial cells, increasing paracellular permeability
(B) It irreversibly bonds to host DNA
(C) It actively replicates on the mucosal surface
(D) It functions as a potent antibiotic against all normal flora

2015

**120.** Identify the INCORRECT statement regarding downstream processing and purification of vaccines.
(A) Centrifugation is frequently used to remove cellular debris
(B) Ultrafiltration concentrates the antigen and removes small molecule impurities
(C) Inactivation agents like formaldehyde are purposefully left in high concentrations in the final product
(D) Chromatography steps purify the antigen based on size, charge, or affinity

2011

**121.** In lyophilization, primary drying occurs under which physical conditions?
(A) Vacuum conditions and low temperature to allow sublimation of ice
(B) High pressure and high heat
(C) Atmospheric pressure and 37°C
(D) Hyperbaric oxygen and sub-zero temperatures

2024

**122.** Under regulatory frameworks, what does the "Master Seed" concept ensure in vaccine production?
(A) That the seed virus/bacteria used for production batches is limited to a strict number of passages to maintain genetic stability
(B) That every batch is made from a freshly isolated wild-type pathogen
(C) That multiple strains are randomly mixed to maximize coverage
(D) That the vaccine cannot be exported to other countries

2011

**123.** A Phase III clinical trial in veterinary vaccinology primarily aims to establish which of the following?
(A) The lethal dose in laboratory mice
(B) Efficacy and safety under natural field conditions involving a large number of target animals
(C) The initial pharmacokinetic clearance rate
(D) The selection of the primary cell line

2025

**124.** The determination of purely specific neutralizing antibodies in post-production in vivo/in vitro potency testing is best assessed using which assay?
(A) Hemagglutination inhibition or Virus Neutralization Test
(B) Western blot
(C) Polymerase chain reaction (PCR)
(D) Total protein quantification

2011

**125.** Read the following statements and choose the correct option:
**Assertion (A):** Edible plant-based vaccines face challenges regarding the induction of oral tolerance.
**Reason (R):** The gut-associated lymphoid tissue (GALT) frequently suppresses systemic immune responses to harmless dietary antigens to prevent food allergies.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2025

**126.** Active immunization provides a distinct advantage over passive immunization due to the generation of what?
(A) Immunological memory
(B) Immediate hypersensitivity
(C) Monoclonal antibodies
(D) Serum sickness

2013

**127.** Which chemical agent is commonly employed to inactivate viruses while preserving the structural integrity of surface epitopes for killed vaccines?
(A) Phenol
(B) Formalin (formaldehyde)
(C) Sodium dodecyl sulfate (SDS)
(D) Concentrated hydrochloric acid

2022

**128.** What is the primary role of T-helper 1 (Th1) cells during the host immune response to a viral pathogen?
(A) They secrete IFN-gamma and IL-2 to activate macrophages and cytotoxic T cells
(B) They secrete IL-4 to drive massive IgE production
(C) They suppress the immune response to prevent tissue damage
(D) They act as professional antigen-presenting cells

2024

**129.** Consider the following statements regarding recombinant subunit vaccines:

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I. They present zero risk of reverting to a virulent state.
II. They consist of isolated proteins or glycoproteins.
III. They usually elicit stronger cellular immunity than live-attenuated vaccines without adjuvants.
IV. They are highly dependent on the correct folding of the antigen for efficacy.

Which of the above statement(s) is/are correct?
(A) I, II, and IV
(B) I and III
(C) II, III, and IV
(D) I, II, and III

2014
**130.** A primary safety concern limiting the widespread veterinary use of replication-competent viral vectors (like certain adenoviruses) is:
(A) They are instantly destroyed by the host complement system
(B) They may cause disease in non-target or immunocompromised animals
(C) They strictly require the use of Freund's Complete Adjuvant
(D) They cannot carry foreign genetic material

2010
**131.** Match the modern genetic manipulation tool in List I with its function in List II:
List I: (Manipulation Tool)
P. CRISPR/Cas9
Q. Reverse genetics
R. Plasmid transfection
S. Cre/loxP system
List II: (Function)
1. Rescue of infectious RNA viruses entirely from cloned cDNA
2. Targeted double-strand breaks for precise gene editing/deletion
3. Introduction of extrachromosomal DNA into eukaryotic cells
4. Site-specific recombination to excise selectable marker genes
(A) P-2, Q-1, R-3, S-4
(B) P-1, Q-2, R-4, S-3
(C) P-4, Q-3, R-1, S-2
(D) P-2, Q-4, R-1, S-3

2003
**132.** In RNA vaccines, the inclusion of modified nucleosides (e.g., pseudouridine) achieves which critical outcome?
(A) It increases the inflammatory response to the RNA
(B) It prevents translation by the host ribosomes
(C) It causes the RNA to immediately degrade
(D) It evades recognition by innate immune sensors (like TLR7) and increases translation efficiency

2001
**133.** Which technique represents a non-tissue-culture-based approach to large-scale antigen production utilizing whole organisms?
(A) Roller bottle culture
(B) Plant-based molecular farming
(C) Hollow fiber bioreactors
(D) Stirred-tank mammalian bioreactors

2018
**134.** Anti-idiotypic vaccines are particularly advantageous when the original target pathogen possesses which characteristic?
(A) It contains a highly mutable RNA genome
(B) It replicates exclusively in the nucleus
(C) It causes rapid host mortality
(D) It possesses highly toxic or difficult-to-purify carbohydrate antigens

2013
**135.** B-cell epitopes mapped for synthetic vaccines are usually classified into two categories based on their structure. What are they?
(A) Linear (continuous) and conformational (discontinuous)
(B) Intracellular and extracellular
(C) Alpha-helical and beta-sheet
(D) Soluble and insoluble

NODIA

2006
**136.** The implementation of DIVA vaccines for classical swine fever (CSF) heavily relies on which specific recombinant subunit antigen to elicit the marker response?
(A) Nucleocapsid protein (N)
(B) Polymerase (P)
(C) Envelope glycoprotein $E_2$
(D) Matrix protein (M)

2025
**137.** Which statement represents the best definition of "pan-genomics" in the context of reverse vaccinology?
(A) The study of the entire core and accessory genomes of all strains within a species
(B) The sequencing of the host immune system genes
(C) The analysis of all RNA transcripts in a single cell
(D) The study of all metabolic byproducts of a

proteins by mammalian ribosomes
(C) RNA vaccines must be transcribed in the nucleus before taking effect
(D) RNA molecules are intrinsically unstable and highly susceptible to ubiquitous RNases

2014
**83.** The process of introducing genetic material into plant cells using Agrobacterium tumefaciens relies on which specific plasmid element?
(A) T-DNA (Transfer DNA)
(B) $ColE_1$ origin
(C) $pUC_{19}$ backbone
(D) SV40 promoter

2006
**84.** Consider the following statements regarding anti-idiotypic vaccines:
I. They consist of antibodies that structurally mimic a pathogenic antigen.
II. They are highly successful and widely commercialized in veterinary medicine today.
III. They are particularly useful for generating immune responses against non-protein antigens (e.g., carbohydrates).
IV. They eliminate the risk of administering a virulent pathogen.
Which of the above statement(s) is/are correct?
(A) I, II, and III
(B) II, III, and IV
(C) I, III, and IV
(D) I and II

2009
**85.** Synthetic peptide vaccines require mapping of T-cell epitopes. T-cell epitopes differ from B-cell epitopes in that they generally must be:
(A) Linear peptides capable of binding to MHC molecules
(B) Complex three-dimensional conformational structures
(C) Large intact protein domains
(D) Glycosylated polysaccharide chains

2011
**86.** Which marker vaccine strategy involves the insertion of a foreign, highly immunogenic tag (like green fluorescent protein) into the vaccine strain?
(A) Negative marker strategy
(B) Subunit deletion strategy
(C) Positive marker strategy
(D) Passive marker strategy

2011
**87.** Reverse vaccinology successfully identified the first effective vaccine targets for which pathogen?
(A) Neisseria meningitidis serogroup B
(B) Mycobacterium bovis
(C) Rabies virus
(D) Foot-and-mouth disease virus

2017
**88.** In structural vaccinology, computationally optimizing an antigen to lock it into a pre-fusion state primarily prevents which event?
(A) Digestion of the antigen by host pepsin
(B) The antigen from binding to the adjuvant
(C) Transcription of the host DNA
(D) Trigger-induced conformational changes that hide neutralizing epitopes

2017
**89.** Which multi-omics approach analyzes the complete set of small-molecule metabolites to assess an animal's physiological response to a vaccine?
(A) Metabolomics
(B) Epigenomics
(C) Transcriptomics
(D) Metagenomics

2024
**90.** Consider the following statements regarding adversomics:
I. It focuses on identifying genetic predispositions to vaccine-associated adverse events.
II. It completely replaces standard Phase I safety testing.
III. It can help design safer vaccines for genetically susceptible subpopulations.
IV. It frequently relies on genome-wide association studies (GWAS).
Which of the above statement(s) is/are correct?
(A) I and III
(B) II
(C) II and IV
(D) I, III, and IV

2009
**91.** Water-in-oil (W/O) emulsions like Freund's Incomplete Adjuvant are highly effective at creating a depot effect but are often restricted in food-producing animals due to which consequence?
(A) Rapid degradation within hours
(B) Suppression of IgG antibody generation
(C) Permanent toxicity of meat for consumption
(D) Severe local tissue site reactions and

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2008

**147.** Under regulatory frameworks, what is a "Certificate of Analysis" (CoA)?
(A) A document proving the vaccine was discovered via reverse vaccinology
(B) A legal document certifying that a specific batch meets all established quality, purity, and potency specifications
(C) The patent filing for the vaccine antigen
(D) The ethical approval for animal testing

2010

**148.** Identify the INCORRECT statement regarding clinical trial phases for veterinary vaccines.
(A) Laboratory efficacy studies must utilize the intended target species
(B) Field trials assess safety and efficacy in animals exposed to natural infection pressure
(C) Pharmacokinetic studies of the antigen are usually as extensive as those required for chemical drugs
(D) Determining the duration of immunity (DoI) is a critical component of efficacy trials

2001

**149.** The in vitro potency assay known as single radial immunodiffusion (SRID) is classically used to quantify which specific viral antigen in influenza vaccines?
(A) Hemagglutinin (HA)
(B) Neuraminidase (NA)
(C) Matrix protein 1 (M1)
(D) Non-structural protein 1 ($NS_1$)

2013

**150.** Read the following statements and choose the correct option:
**Assertion (A):** Plant-based edible vaccines circumvent the need for standard cold-chain logistics.
**Reason (R):** The antigens expressed in plant tissues like seeds or tubers are often protected from thermal degradation by the natural rigid cell wall and compartmentalization.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2011

**151.** Which factor represents the most critical barrier to the success of orally administered live-attenuated vaccines?
(A) The harsh acidic environment of the stomach and proteolytic enzymes in the gut
(B) They induce an overwhelmingly strong systemic IgG response
(C) They bypass the gut-associated lymphoid tissue entirely
(D) They require co-administration with high doses of antibiotics

2025

**152.** Toxoid vaccines are highly effective against which class of bacterial pathogens?
(A) Pathogens that rely exclusively on intracellular replication
(B) Pathogens whose primary pathogenesis is mediated by secreted exotoxins (e.g., Clostridium tetani)
(C) Gram-negative bacteria acting solely via endotoxin (LPS) shock
(D) Obligate intracellular rickettsial organisms

NODIA

2018

**153.** A robust CD8+ T cell response is most effectively induced when the vaccine antigen is processed through which cellular pathway?
(A) Exogenous pathway and presented on MHC Class II
(B) Endogenous (cytosolic) pathway and presented on MHC Class I
(C) Lysosomal degradation without MHC presentation
(D) Direct binding to B-cell receptors

2001

**154.** In the development of recombinant subunit vaccines, what is a primary limitation of using Saccharomyces cerevisiae (baker's yeast) as an expression system?
(A) Inability to grow in liquid culture
(B) Hyper-mannosylation of glycoproteins, which may alter antigenicity
(C) Complete lack of endoplasmic reticulum
(D) Production of deadly exotoxins

2003

**155.** Match the recombinant vaccine platform in List I with its clinical veterinary example in List II:
List I: (Platform)
P. Recombinant Poxvirus vector
Q. DIVA subunit vaccine
R. Live gene-deletion mutant
S. DNA vaccine
List II: (Example)
1. gE-deleted BoHV-1 vaccine for infectious bovine rhinotracheitis
2. Rabies glycoprotein expressed in Vaccinia

adverse event monitoring
3. Initial safety and immunogenicity assessment in a small group of target animals
4. Dose determination, schedule optimization, and preliminary efficacy in challenge models

(A) P-3, Q-4, R-1, S-2
(B) P-1, Q-2, R-3, S-4
(C) P-3, Q-1, R-4, S-2
(D) P-4, Q-3, R-2, S-1

2023

**174.** During quality control testing, the "in vivo potency test" typically involves which procedure?
(A) Mixing the vaccine with serum in a test tube and observing for precipitation
(B) Injecting the vaccine into a mass spectrometer
(C) Measuring the electrical conductivity of the vaccine vial
(D) Vaccinating a group of animals and subsequently challenging them with a virulent strain to measure protection

2009

**175.** Read the following statements and choose the correct option:
**Assertion (A):** The use of unmethylated CpG motifs in DNA vaccines significantly enhances their immunogenicity.
**Reason (R):** CpG motifs are recognized by Toll-Like Receptor 9 (TLR9), leading to potent activation of the innate immune system and promoting Th1 responses.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2011

**176.** Which type of vaccine induces an immune response that most closely mimics the response generated by natural infection?
(A) Live-attenuated vaccines
(B) Inactivated (killed) vaccines
(C) Toxoid vaccines
(D) Synthetic peptide vaccines

2021

**177.** When considering traditional vaccine platforms, what is the primary risk associated with inadequately inactivated "killed" viral vaccines?
(A) They may cause an outbreak of the actual disease
(B) They induce an overwhelmingly powerful mucosal response
(C) They spontaneously turn into DNA vaccines
(D) They become edible

2010

**178.** What determines the specific isotype (e.g., switching from IgM to IgG or IgA) of an antibody produced by a B cell during an immune response?
(A) The physical size of the antigen
(B) Cytokine signals from T-helper cells orchestrating class switch recombination
(C) The type of adjuvant directly binding to the B cell
(D) The route of administration exclusively

2010

**179.** Consider the following statements regarding Poxvirus vectors used in veterinary vaccines:
I. They can accommodate large insertions of up to 25 kb of foreign DNA.
II. They replicate in the cytoplasm of the host cell.
III. They possess a very high risk of integrating into the host nucleus.
IV. Recombinant canarypox vectors undergo an abortive infection in mammalian hosts, ensuring safety.
Which of the above statement(s) is/are correct?
(A) I, II, and IV
(B) II, III, and IV
(C) I, II, and III
(D) I and IV

2021

**180.** A rationally attenuated bacterial vaccine is created by knocking out the aroA gene. The organism becomes dependent on the host for which essential nutrients that mammals cannot provide?
(A) Aromatic amino acids (e.g., tryptophan, tyrosine, phenylalanine)
(B) Glucose and lactose
(C) Essential fatty acids
(D) Iron and zinc

2021

**181.** In DNA vaccine constructs, the presence of a polyadenylation (poly-A) signal sequence serves what crucial biological function?
(A) It stabilizes the transcribed mRNA and facilitates its export from the nucleus for translation
(B) It promotes the integration of the plasmid into the host chromosome

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(A) A specific immune response parameter that is statistically associated with protection against clinical disease
(B) A geographical area where the disease is absent
(C) The chemical bond between an antigen and adjuvant
(D) The legal patent protecting the vaccine formulation

2013
**165.** The field of adversomics would most likely investigate a link between an adverse vaccine reaction and a single nucleotide polymorphism (SNP) in which type of gene?
(A) A gene regulating basic cellular glycolysis
(B) A gene coding for hair color
(C) A gene encoding an innate immune sensor, such as a Toll-Like Receptor (TLR)
(D) A gene for viral reverse transcriptase

2011
**166.** Aluminum salts (alum) are the most widely used adjuvants in veterinary medicine. They are notably poor at inducing which type of immune response?
(A) Th2-biased responses
(B) IgG production
(C) Cytotoxic CD8+ T cell (Th1) responses
(D) B-cell memory

2006
**167.** Identify the INCORRECT statement regarding the use of cytokines as biological vaccine enhancers.
(A) Short in vivo halF-lives limit their free protein efficacy
(B) Effective delivery via incorporation into viral vectors
(C) No potential risk for systemic toxicity or cytokine storm
(D) IL-12 is often used to skew the response towards Th1

2025
**168.** PLGA (poly-lactic-co-glycolic acid) microspheres degrade in the host body primarily through which chemical process?
(A) Hydrolysis of their ester linkages
(B) Enzymatic digestion by pepsin
(C) Oxidation by reactive oxygen species
(D) Photodegradation by UV light

2023
**169.** What gives Virus-Like Particles (VLPs) their highly potent immunogenicity compared to soluble monomeric proteins?
(A) Their ability to replicate inside host cells
(B) The repetitive, high-density display of conformational epitopes on a geometric surface
(C) Their intrinsic toxicity to antigen-presenting cells
(D) Their total lack of recognizable epitopes

2008
**170.** During large-scale bioreactor cultivation of mammalian cells for viral vaccine production, which parameter is routinely monitored to assess cell growth and metabolic activity?
(A) Lactate and glucose concentrations in the media
(B) Light transmission through the reactor walls
(C) The magnetic field generated by the impeller
(D) The atmospheric pressure outside the reactor

2015
**171.** In a lyophilized vaccine formulation, what is the specific role of a "bulking agent" like mannitol?
(A) To provide an elegant, structurally sound cake appearance and physical support
(B) To destroy adventitious viruses
(C) To act as the primary antigen
(D) To dramatically lower the pH

2016
**172.** Under pharmacopeial guidelines, what does the "reversion to virulence" test demonstrate?
(A) That a killed vaccine contains no live particles
(B) That a live-attenuated vaccine strain does not become pathogenic after repeated serial passages in the target host
(C) That the adjuvant does not cause cancer
(D) That the vaccine can cure an already infected animal

2017
**173.** Match the clinical trial phase in List I with its primary objective in List II:
List I: (Clinical Trial Phase)
P. Phase I
Q. Phase II
R. Phase III
S. Phase IV
List II: (Objective)
1. Large-scale field efficacy and safety monitoring under natural conditions
2. Post-marketing surveillance and long-term

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(D) The immediate exocytosis of all nucleic acids by macrophages

2017

**207.** In plant-based edible vaccines, the concept of "bioencapsulation" refers to what phenomenon?
(A) Surrounding the plant seed with a thick plastic shell
(B) The plant cell wall protecting the expressed antigen from rapid degradation in the stomach
(C) Genetically modifying the plant to produce a liposome
(D) Extracting the antigen and placing it in a gelatin capsule

2025

**208.** Consider the following statements regarding the spatial conformation of synthetic peptide vaccines:
I. Small linear peptides often lack a rigid 3D structure in solution.
II. Loss of native conformation limits the induction of high-affinity neutralizing antibodies against conformational epitopes.
III. Cyclization is a strategy used to constrain the peptide into a rigid, native-like shape.
Which of the above statement(s) is/are correct?
(A) I and II
(B) II and III
(C) I and III
(D) I, II, and III

2024

**209.** The companion diagnostic for a DIVA marker vaccine must be highly specific to avoid what major programmatic failure?
(A) False positives, which would lead to the unnecessary culling of healthy vaccinated animals
(B) Total destruction of the vaccine vial
(C) Cross-reactivity with the adjuvant
(D) Induction of auto-immune disease

2002

**210.** Which computational tool is an integral part of reverse vaccinology for predicting MHC Class I and II binding epitopes?
(A) NetMHC / IEDB tools
(B) Autodock Vina
(C) PyMOL
(D) AlphaFold

2022

**211.** The application of AlphaFold, an AI system that predicts 3D protein structures from amino acid sequences, has profoundly accelerated which field of vaccinology?
(A) Structural vaccinology
(B) Reverse vaccinology
(C) Adversomics
(D) Quality control testing

2015

**212.** In systems vaccinology, determining the "transcriptomic signature" of a highly effective vaccine shortly after administration allows researchers to do what?
(A) Predict the final antibody titer without waiting months for the serological response
(B) Bypass the need for sterilizing the bioreactor
(C) Eliminate all need for clinical safety trials
(D) Instantly cure the animal of any existing infection

2025

**213.** Determine the correct order of the biological cascade leading to an adverse vaccine reaction mediated by IgE (anaphylaxis).
(A) First exposure to antigen, IgE production, IgE binds to mast cells/basophils, second exposure cross-links IgE, degranulation (histamine release)
(B) First exposure to antigen, IgE binds to mast cells, degranulation, second exposure, IgE production
(C) IgE production, first exposure to antigen, degranulation, second exposure, IgE binds to mast cells
(D) First exposure, degranulation, IgE production, second exposure, IgE binds to mast cells

NODIA

2019

**214.** Which of the following adjuvants is characterized by the presence of killed mycobacteria within a water-in-oil emulsion to provide a powerful stimulus for cell-mediated immunity?
(A) Freund's Complete Adjuvant (FCA)
(B) Freund's Incomplete Adjuvant (FIA)
(C) Aluminum hydroxide gel
(D) QS-21 saponin

2001

**215.** The cytokine Interferon-gamma (IFN-g) acts as a powerful immunomodulator. What is its principal source during the early phases of the immune response?
(A) Natural Killer (NK) cells and Th1 cells
(B) B lymphocytes and plasma cells
(C) Erythrocytes

(C) It acts as the promoter binding site for RNA polymerase
(D) It degrades the plasmid after a set time

2018
**182.** Which of the following is a significant biosafety advantage of plant expression system-based vaccines (molecular farming)?
(A) They harbor zero risk of contamination with mammalian viral pathogens or prions
(B) They completely eliminate the need for host immune cells
(C) They replicate vigorously in the gastrointestinal tract
(D) They bypass all regulatory approval frameworks

2002
**183.** Identify the INCORRECT statement regarding the Jerne immune network theory and anti-idiotypic vaccines.
(A) An idiotype is a unique set of antigenic determinants located on the variable region of an antibody
(B) Anti-idiotypic vaccines are particularly well-suited for easily cultivated, highly safe protein antigens
(C) Ab2 antibodies structurally mimic the original antigen
(D) The strategy relies on inducing an Ab3 response in the patient

2023
**184.** In synthetic peptide vaccines, what is the primary role of a "spacer" or "linker" sequence placed between multiple distinct epitopes?
(A) To prevent the epitopes from interfering with each other's independent folding and recognition
(B) To make the peptide highly toxic to bacteria
(C) To act as a direct adjuvant binding site for TLRs
(D) To ensure the peptide rapidly degrades in the blood

2006
**185.** Determine the correct sequence of steps involved in developing a DIVA vaccine and companion diagnostic strategy.
(A) Identify a non-essential highly immunogenic viral protein, delete the gene encoding this protein from the vaccine strain, develop an ELISA targeting the deleted protein, validate the ELISA to distinguish infected from vaccinated animals
(B) Develop an ELISA, identify a non-essential protein, validate the ELISA, delete the gene
(C) Delete the gene, develop an ELISA, identify a non-essential protein, validate the ELISA
(D) Identify a non-essential protein, develop an ELISA, delete the gene, validate the ELISA

2002
**186.** During the in silico screening phase of reverse vaccinology, an ideal novel vaccine candidate antigen should possess which characteristic?
(A) High homology with host proteins to avoid rejection
(B) High sequence conservation across multiple virulent strains
(C) Strict intracellular localization within the pathogen
(D) Absence of any transmembrane domains or signal sequences

2024
**187.** Structural vaccinology utilizes X-ray crystallography to determine the 3D structure of an antigen. What must be achieved first before crystallography can be performed?
(A) Denaturation of the protein using urea
(B) High-yield purification and generation of a stable, ordered protein crystal
(C) Cleavage of the protein into single amino acids
(D) Attachment of the protein to a heavy metal adjuvant

2019
**188.** Systems vaccinology uses mathematical modeling to integrate multi-omics data. What is the ultimate predictive goal of this approach?
(A) Determining the color of the final vaccine vial
(B) Forecasting the robustness and longevity of the immune response based on early molecular signatures
(C) Predicting the evolutionary origin of the host species
(D) Calculating the exact manufacturing cost per dose

2006
**189.** In the context of adversomics, which hypersensitivity reaction type is associated with immune complex deposition (e.g., Arthus reaction) following hyper-immunization?
(A) Type I
(B) Type II
(C) Type III

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virus (Raboral V-RG)
3. West Nile Virus plasmid vaccine for horses
4. $E_2$ glycoprotein marker vaccine for Classical Swine Fever

(A) P-2, Q-4, R-1, S-3
(B) P-4, Q-2, R-1, S-3
(C) P-1, Q-4, R-2, S-3
(D) P-2, Q-1, R-4, S-3

2002
**156.** The creation of a "rationally attenuated" bacterial vaccine strain, such as a Salmonella mutant lacking the adenylate cyclase gene (cya), ensures safety through which mechanism?
(A) The bacteria exhibit impaired survival and replication in host tissues due to metabolic deficiency
(B) The bacteria cannot synthesize a cell wall
(C) The bacteria cannot secrete toxins
(D) The bacteria become strictly anaerobic

2005
**157.** Consider the following statements regarding DNA vaccines:
I. They consist of a bacterial plasmid carrying the antigen gene.
II. They pose a high risk of causing full clinical disease.
III. They are highly thermostable compared to live viral vaccines.
IV. They are routinely administered via a "gene gun" or electroporation.
Which of the above statement(s) is/are correct?
(A) I, II, and III
(B) I, III, and IV
(C) II, III, and IV
(D) I and IV

2009
**158.** Agrobacterium-mediated transformation in plant vaccinology relies on the virulence (vir) genes of the Ti plasmid. What is the precise function of these vir genes?
(A) They encode the vaccine antigen directly
(B) They mediate the excision, transfer, and integration of the T-DNA into the plant genome
(C) They provide resistance to herbicides
(D) They cause rapid necrosis of the plant leaves

2012
**159.** When an anti-idiotypic antibody (Ab2) acts as an "internal image" of a pathogen's antigen, it is capable of stimulating the production of which antibody type in the vaccinated host?
(A) Ab1
(B) Ab3 (which neutralizes the actual pathogen)
(C) Ab4
(D) Natural killer cell receptors

2019
**160.** Determine the correct sequence for mapping B-cell epitopes for a synthetic peptide vaccine.
(A) Synthesize overlapping peptides derived from the antigen sequence, screen peptides with neutralizing sera, identify strongly binding peptide fragments, optimize peptide sequence and conformation
(B) Screen peptides with neutralizing sera, synthesize overlapping peptides, optimize sequence, identify fragments
(C) Optimize sequence, synthesize overlapping peptides, screen with sera, identify fragments
(D) Identify fragments, optimize sequence, screen with sera, synthesize peptides

2002
**161.** A DIVA strategy is deployed in an avian influenza outbreak. If the vaccine uses the $H_5$ hemagglutinin and an $N_1$ neuraminidase ($H_5N_1$), but the wild-type circulating virus is $H_5N_2$, what serves as the negative marker?
(A) Antibodies against $H_5$
(B) Antibodies against $N_2$
(C) Antibodies against $N_1$
(D) Matrix protein antibodies

2013
**162.** Reverse vaccinology relies heavily on the principle that the most effective vaccine targets (antigens) are typically located where on the bacterial cell?
(A) Surface-exposed or actively secreted
(B) Deeply buried within the nucleoid
(C) Bound exclusively to the inner face of the plasma membrane
(D) Within the ribosomal subunits

2005
**163.** Structural vaccinology often targets the "fusion machinery" of enveloped viruses. What triggers the conformational change of these fusion proteins during natural infection?
(A) Binding to a host cell receptor and/or changes in endosomal pH
(B) Exposure to host ribosomes
(C) Contact with host cell mitochondria
(D) Cleavage by viral polymerase

2023
**164.** In the context of Vaccinomics, a "correlate of protection" is defined as:

NODIA

**Reason (R):** Real-time PCR accurately quantifies the viral genome copy number, ensuring the batch contains the correct infectious dose and meets identity standards.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2018

**199.** What distinguishes a primary immune response from a secondary immune response regarding the affinity of the antibodies produced?

(A) Primary response antibodies generally have higher affinity due to somatic hypermutation
(B) Secondary response antibodies have progressively higher affinity due to affinity maturation
(C) Primary response antibodies are strictly monoclonal with perfect affinity
(D) There is no difference in affinity, only in quantity

2010

**200.** Which type of immunity is transferred to an embryo/fetus via the placenta in species with hemochorial placentation, but NOT in species with epitheliochorial placentation?

(A) Natural passive immunity (maternal IgG)
(B) Artificial active immunity
(C) Natural active immunity
(D) Artificial passive immunity

2016

**201.** Which chemical is historically favored for the creation of toxoid vaccines due to its ability to cross-link proteins and destroy toxin activity without eliminating immunogenicity?

(A) Formaldehyde
(B) Ethanol
(C) Hydrogen peroxide
(D) Sodium hypochlorite

2008

**202.** Match the T-cell subset in List I with its primary function in the host immune response in List II:

List I: (T-cell Subset)
P. Th1 cells
Q. Th2 cells
R. T follicular helper (Tfh) cells
S. Regulatory T cells (Treg)

List II: (Primary Function)
1. Drive B-cell activation, class switching, and affinity maturation in germinal centers
2. Support macrophage activation and cell-mediated immunity against intracellular pathogens
3. Promote eosinophil recruitment and helminth immunity (IgE)
4. Suppress excessive immune responses and maintain tolerance

(A) P-2, Q-3, R-1, S-4
(B) P-1, Q-2, R-3, S-4
(C) P-3, Q-1, R-4, S-2
(D) P-2, Q-1, R-4, S-3

2024

**203.** The fundamental challenge of expressing a heavily glycosylated viral envelope protein in an E. coli system is:

(A) E. coli strictly requires mammalian serum to grow
(B) E. coli lacks the cellular machinery for eukaryotic N- and O-linked glycosylation
(C) E. coli actively digests all foreign viral RNA
(D) E. coli produces a cell wall that binds the protein irreversibly

2011

**204.** Recombinant Salmonella vectors are typically administered via which route to capitalize on their natural tropism?

(A) Oral administration
(B) Intramuscular injection
(C) Intravenous injection
(D) Subcutaneous injection

2019

**205.** Identify the INCORRECT statement regarding targeted gene-deletion mutants.

(A) They are created using precise molecular techniques like homologous recombination
(B) They can serve as excellent DIVA vaccines
(C) They are considered rationally attenuated
(D) They spontaneously regenerate the deleted gene when introduced into the host

2006

**206.** When formulating a DNA vaccine, the efficiency of cellular uptake and subsequent nuclear import is severely restricted by what biological barrier?

(A) The negative charge of the plasmid repelling the negatively charged cell membrane, and physical degradation by nucleases
(B) The high positive charge of the plasmid DNA repelling the cell membrane
(C) The rapid crystallization of DNA at body temperature

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(D) Two vaccines given at the same time spontaneously recombine

2019

**225.** Match the immune response component in List I with its kinetic characteristic in List II:

List I: (Immune Response Component)

P. Primary IgM response

Q. Primary IgG response

R. Secondary IgG response

S. Memory T cell response

List II: (Kinetic Characteristic)

1. Exhibits a rapid, massive clonal expansion upon re-exposure to the antigen
2. Peaks early and rapidly declines
3. Develops more slowly, peaks later, and dominates the primary systemic response
4. Is immediate, reaches much higher titers, and demonstrates affinity maturation

(A) P-2, Q-3, R-4, S-1

(B) P-3, Q-2, R-1, S-4

(C) P-4, Q-3, R-1, S-2

(D) P-1, Q-4, R-2, S-3

2024

**226.** What is the fundamental mechanism by which a DNA vaccine successfully elicits a CD8+ Cytotoxic T Lymphocyte (CTL) response?

(A) By remaining in the extracellular fluid and cross-linking B-cell receptors

(B) By transfecting host cells which synthesize the antigen in the cytosol, leading to proteasomal processing and MHC Class I presentation

(C) By directly binding to the T-cell receptor without MHC

(D) By causing massive necrosis that nonspecifically activates T cells

2002

**227.** Consider the following statements regarding recombinant bacterial vectors (e.g., Listeria monocytogenes):

I. They are strictly extracellular pathogens.

II. Listeria naturally escapes the phagosome into the host cytosol, effectively driving potent CD8+ T cell responses.

III. Attenuation is often achieved by deleting essential virulence genes, like actA.

Which of the above statement(s) is/are correct?

(A) I and II

(B) II and III

(C) I and III

(D) I, II, and III

2010

**228.** A rationally attenuated viral vaccine, lacking a specific immune-evasion gene, is generally superior to a classically attenuated virus because:

(A) The genetic basis of attenuation is defined and the risk of back-mutation to wild-type is virtually eliminated

(B) It does not require cold-chain storage

(C) It can replicate without using host cell ribosomes

(D) It inherently acts as its own companion diagnostic test

2014

**229.** Plasmid DNA vaccines frequently contain unmethylated CpG motifs. Which viral promoter is ubiquitous in these constructs?

(A) SV40 or human Cytomegalovirus (hCMV) immediate-early promoter

(B) $T_7$ bacteriophage promoter

(C) Cauliflower Mosaic Virus (CaMV) 35S promoter

(D) Lac operon promoter

NODIA

2010

**230.** Determine the correct sequence for producing a synthetic peptide vaccine.

(A) Identify the protective epitope, chemically synthesize the peptide, conjugate the peptide to a carrier protein, mix with an appropriate adjuvant

(B) Conjugate to a carrier protein, identify the protective epitope, chemically synthesize the peptide, mix with adjuvant

(C) Mix with adjuvant, identify the protective epitope, chemically synthesize the peptide, conjugate to a carrier protein

(D) Chemically synthesize the peptide, mix with adjuvant, identify the protective epitope, conjugate to a carrier

2001

**231.** The primary challenge with anti-idiotypic vaccines (Ab2) acting as internal images is that they:

(A) May induce a strong immune response against the host's own immunoglobulins, potentially leading to autoimmunity

(B) Are universally lethal to the host

(C) Entirely lack any peptide bonds

(D) Can only be manufactured in plants

2011

**232.** Synthetic peptide vaccines typically require coupling to a carrier protein because:

(A) Small peptides act as haptens and fail to

(D) Endothelial cells

2021

**216.** Consider the following statements regarding particulate delivery systems:

I. Liposomes can protect antigens from proteolytic degradation.

II. ISCOMs strictly require the use of Freund's Complete Adjuvant to function.

III. Particulate systems often enhance uptake by antigen-presenting cells because they mimic the size and dimensions of pathogens.

Which of the above statement(s) is/are correct?

(A) I and II
(B) II and III
(C) I and III
(D) I

2012

**217.** Targeted mucosal delivery of vaccines utilizing M-cell targeted ligands aims to exploit the transcytosis capacity of M-cells located where?

(A) In the follicle-associated epithelium of Peyer's patches and NALT
(B) In the glomerulus of the kidney
(C) In the stratum corneum of the skin
(D) In the choroid plexus of the brain

2014

**218.** When optimizing large-scale bioreactor parameters for suspension cell cultures, monitoring and controlling "shear stress" is vital. Why?

(A) High shear stress causes the media to freeze
(B) Excessive shear forces generated by aggressive agitation can physically rupture and kill mammalian cells
(C) Shear stress spontaneously mutates the viral genome
(D) Low shear stress degrades the adjuvant

2002

**219.** During the formulation process, excipients known as "surfactants" (e.g., Polysorbate 80) are included primarily to prevent what physical degradation mechanism?

(A) Protein aggregation and surface adsorption
(B) Hydrolysis
(C) Oxidation
(D) Deamidation

2009

**220.** An expiry date for a veterinary vaccine is established based primarily on which type of testing?

(A) Real-time and accelerated stability testing
(B) Challenge testing in pregnant animals
(C) Genomic sequencing of the final product
(D) Mass spectrometry of the adjuvant

2015

**221.** In a clinical trial context, "immunogenicity" specifically refers to:

(A) The capacity of the vaccine to stimulate a measurable humoral and/or cellular immune response
(B) The physical stability of the vaccine formulation
(C) The ability of the vaccine to cause fever
(D) The speed at which the vaccine degrades in sunlight

2019

**222.** Read the following statements and choose the correct option:

**Assertion (A):** In vitro potency tests are increasingly replacing in vivo challenge models for batch release quality control.

**Reason (R):** In vitro assays, such as antigen quantification via ELISA, are faster, more reproducible, and align with animal welfare standards.

(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2010

**223.** Identify the INCORRECT statement regarding passive immunization.

(A) It relies on the administration of pre-formed specific antibodies
(B) It provides immediate protection post-administration
(C) It establishes long-lasting immunological memory
(D) Equine tetanus antitoxin is a classic example

2007

**224.** Traditional live-attenuated vaccines often face the problem of "interference." What does this term mean in a clinical veterinary setting?

(A) Maternal antibodies neutralize the live vaccine virus before it can replicate and induce an immune response
(B) The vaccine interferes with the absorption of dietary nutrients
(C) The vaccine adjuvant destroys the viral antigen

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(D) Type IV

2003

**190.** Match the advanced particulate delivery system in List I with its unique characteristic in List II:
List I: (Delivery System)
P. Liposomes
Q. PLGA nanoparticles
R. VLPs
S. Nanogels
List II: (Characteristic)
1. Spontaneously assembled viral proteins lacking genomes
2. Phospholipid bilayer vesicles capable of encapsulating hydrophilic and lipophilic antigens
3. Swellable, cross-linked hydrophilic polymer networks responsive to physiological triggers
4. Biodegradable polyesters providing controlled, sustained antigen release
(A) P-2, Q-4, R-1, S-3
(B) P-4, Q-2, R-1, S-3
(C) P-1, Q-4, R-2, S-3
(D) P-2, Q-3, R-1, S-4

2016

**191.** TLR agonists are utilized as modern adjuvants. Poly(I:C) is a synthetic analog of double-stranded RNA. Which specific Toll-Like Receptor does it activate?
(A) $TLR_2$
(B) $TLR_3$
(C) $TLR_4$
(D) $TLR_5$

2003

**192.** Which chemokine would be most rationally incorporated into a vaccine to attract dendritic cells and macrophages to the injection site?
(A) CCL2 (MCP-1)
(B) CXCL8 (IL-8)
(C) Interleukin-10
(D) TGF-beta

2016

**193.** The primary reason for using a targeted mucosal delivery method for a respiratory pathogen vaccine is to establish:
(A) Local mucosal immunity mediated by secretory IgA and tissue-resident memory T cells
(B) Sterilizing immunity in the bloodstream
(C) A strong depot effect in the muscle
(D) Rapid degradation of the antigen in the stomach

2007

**194.** In scaling up vaccine production, the term "downstream processing" refers strictly to which set of activities?
(A) The initial isolation of the wild-type virus
(B) The harvesting, purification, concentration, and formulation of the product
(C) The cultivation of cells in the bioreactor
(D) The clinical trial testing in target animals

2002

**195.** Consider the following statements regarding vaccine formulation and stabilizers:
I. Stabilizers protect the antigen from physical and chemical degradation during storage.
II. Gelatin and human serum albumin are historic stabilizers that carry risks of hypersensitivity or adventitious agents.
III. Stabilizers are actively removed from the vaccine immediately prior to injection.
IV. Sugars like trehalose and sucrose are commonly used highly safe stabilizers.
Which of the above statement(s) is/are correct?
(A) I, II, and IV
(B) II, III, and IV
(C) I and III
(D) I, II, and III

NODIA

2001

**196.** Under pharmacopeial guidelines, what does the "sterility test" of a final vaccine batch confirm?
(A) The absolute absence of any living bacteria or fungi
(B) The absence of the vaccine antigen
(C) The inability of the vaccinated animal to reproduce
(D) The absence of heavy metals

2001

**197.** During pre-clinical efficacy trials, what is the significance of establishing a "challenge model"?
(A) It determines if the vaccine causes pain upon injection
(B) It evaluates if the induced immune response protects the animal against deliberate infection with a virulent pathogen
(C) It measures the maximum volume that can be injected
(D) It screens out animals with genetic defects

2014

**198.** Read the following statements and choose the correct option:
**Assertion (A):** Real-time PCR is frequently utilized in the post-production quality control of live-attenuated vaccines.

reliably recruit T-cell help without the carrier's T-cell epitopes
(B) Naked peptides are intensely toxic to the liver
(C) Peptides dissolve instantly in air
(D) The carrier protein contains the actual neutralizing epitope

2015
**233.** In the context of DIVA strategies, an "indirect ELISA" used to detect natural infection relies on coating the assay plate with what?
(A) The purified marker antigen that is present in the wild-type virus but deleted from the vaccine
(B) The whole wild-type virus
(C) Monoclonal antibodies against the host species
(D) The vaccine adjuvant

2010
**234.** Reverse vaccinology utilizes the concept of the "pan-genome." What does the "core genome" specifically represent in this context?
(A) Genes shared by all sequenced strains of a particular bacterial species
(B) Genes present in only a single virulent strain
(C) Genes encoded exclusively on plasmids
(D) Host genes that interact with the bacteria

2025
**235.** Which structural mechanism allows computationally designed pre-fusion stabilized antigens to generate a superior neutralizing antibody response?
(A) They expose highly conserved, neutralization-sensitive epitopes that are otherwise hidden after the protein undergoes conformational rearrangement
(B) They present completely denatured, linear sequences to the immune system
(C) They actively destroy host B cells
(D) They possess zero tertiary structure

2023
**236.** The integration of genomic, transcriptomic, and proteomic data to model the entire host response to a vaccine is termed:
(A) Reverse vaccinology
(B) Structural biology
(C) Systems vaccinology
(D) Epidemiology

2023
**237.** Adversomics aims to identify biomarkers that predict severe adverse vaccine reactions. Which host genetic complex is overwhelmingly implicated in such idiosyncratic responses?
(A) The Major Histocompatibility Complex (MHC)
(B) The mitochondrial genome
(C) The hemoglobin gene locus
(D) The keratin gene cluster

2018
**238.** What is the fundamental difference between an adjuvant and an antigen?
(A) An antigen specifically directs the adaptive immune response, while an adjuvant non-specifically enhances the magnitude and type of that response
(B) An adjuvant specifically directs the adaptive response, while an antigen non-specifically enhances it
(C) Adjuvants are always proteins; antigens are always lipids
(D) Antigens are used strictly in live vaccines; adjuvants strictly in killed vaccines

NODIA

2002
**239.** The incorporation of a cytokine like Interleukin-4 (IL-4) into a vaccine construct would bias the immune response towards which profile?
(A) Th2 and robust humoral (antibody) production
(B) Th1 and Cytotoxic T-cells
(C) T regulatory cell suppression
(D) Macrophage hyperactivation

2025
**240.** Match the nanotechnology platform in List I with its structural definition in List II:
List I: (Nanotechnology Platform)
P. Liposome
Q. Virosome
R. VLP (Virus-Like Particle)
S. Polymeric Nanoparticle
List II: (Structural Definition)
1. Empty, multiprotein structures mimicking viral organization without the genome
2. A liposome incorporating functionally active viral envelope glycoproteins
3. Solid matrix particles made of biodegradable polymers like PLGA
4. Spherical vesicles composed of one or more phospholipid bilayers
(A) P-4, Q-2, R-1, S-3
(B) P-2, Q-4, R-1, S-3
(C) P-1, Q-3, R-2, S-4
(D) P-4, Q-1, R-2, S-3

# ANSWERS

**1.** (B) It provides immediate but temporary humoral protection
Passive immunization involves the direct transfer of pre-formed antibodies to a recipient, providing immediate immunological protection against pathogens or toxins. This method is particularly advantageous in emergency situations where there is insufficient time for the host to mount an active immune response. However, the protection is temporary because the transferred antibodies eventually degrade without memory.

**2.** (D) Repeated serial passage in non-natural host cell cultures
Traditional live viral vaccines are frequently attenuated by repeatedly passing the virus through non-natural host cells or embryos. This process forces the pathogen to adapt to the new environment, causing it to accumulate mutations that reduce its virulence in the original natural host. Consequently, the virus replicates enough to induce immunity without causing clinical disease.

**3.** (A) Immunoglobulin M
During the initial encounter with a pathogen, the primary humoral immune response follows a specific kinetic profile. Immunoglobulin M is the first isotype to be secreted by activated B cells and typically reaches its peak concentration before other classes. This early response is essential for immediate neutralization and complement activation before higher-affinity antibodies like IgG are produced.

**4.** (C) I and III
Prokaryotic systems like bacteria lack the complex cellular machinery required for essential post-translational modifications such as glycosylation. Because these subunit vaccines consist of isolated proteins rather than live pathogens, they cannot revert to virulence. Furthermore, these purified antigens are often less immunogenic than whole-agent vaccines, necessitating the use of potent adjuvants to stimulate a robust immune response.

**5.** (A) They can accommodate large insertions of foreign DNA
Poxviruses are distinguished by their exceptionally large double-stranded DNA genomes, which provide significant capacity for the insertion of multiple foreign genetic sequences. This characteristic allows them to serve as versatile vaccine vectors capable of expressing several antigens simultaneously. Their replication in the host cytoplasm further enhances their safety profile by avoiding potential integration into the cellular genome.

**6.** (D) A non-essential virulence or immunomodulatory gene
Rationally attenuated vaccines are developed by precisely deleting specific genes that contribute to the pathogen's ability to cause disease or evade the immune system. By targeting non-essential virulence or immunomodulatory genes, the pathogen remains viable and capable of replication but is significantly weakened. This approach ensures that the vaccine cannot cause illness while maintaining its immunogenic properties.

**7.** (B) Cytomegalovirus (CMV) early promoter
In DNA vaccine constructs, the selection of a strong regulatory element is vital for ensuring high levels of antigen production. The cytomegalovirus early promoter is widely utilized because it drives constitutive and robust gene expression across a broad range of mammalian cell types. This high-level expression is necessary to provide sufficient protein for effective processing and immune recognition.

**8.** (A) Gene insertion into T-DNA, Agrobacterium infection of plant tissue, integration into plant genome, expression of antigen
Producing plant-based vaccines using Agrobacterium tumefaciens involves a specific chronological sequence of molecular events. First, the desired antigen gene is inserted into the transfer DNA region of a plasmid. Then, the bacteria infect the plant tissue, allowing the transfer DNA to integrate into the plant genome. Finally, the plant cells utilize their own machinery to express the antigen.

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sIgA compared to intranasal vaccines.

Which of the above statement(s) is/are correct?

(A) I and II
(B) II and III
(C) I and III
(D) I, II, and III

2007

**250.** Match the vaccine type in List I with its defining characteristic in List II:

List I: (Vaccine Type)

P. Subunit vaccine
Q. Toxoid vaccine
R. Live-attenuated vaccine
S. Inactivated whole-agent vaccine

List II: (Defining Characteristic)

1. Comprises an entire pathogen chemically or physically killed
2. Contains a weakened form of the pathogen capable of limited replication
3. Comprises isolated, purified structural components of a pathogen
4. Comprises a chemically detoxified pathogenic exotoxin

(A) P-3, Q-4, R-2, S-1
(B) P-4, Q-3, R-1, S-2
(C) P-2, Q-1, R-4, S-3
(D) P-1, Q-2, R-3, S-4

2018

**251.** Which factor strictly limits the size of the foreign gene insert in recombinant Adenovirus vaccine vectors?

(A) The physical packaging constraints of the icosahedral viral capsid
(B) The amount of ATP available in the host cell
(C) The speed of the host ribosome
(D) The lipid composition of the viral envelope

2009

**252.** The engineering of a bacterial pathogen to delete the genes responsible for synthesizing its lipopolysaccharide (LPS) O-antigen side chain creates a "rough" mutant. What is the primary purpose of this rational attenuation?

(A) To create a negative marker DIVA vaccine strain that lacks the immunodominant O-antigen
(B) To make the bacteria highly resistant to antibiotics
(C) To increase the toxicity of the endotoxin
(D) To allow the bacteria to evade the immune system indefinitely

2017

**253.** In DNA vaccine technology, what is the role of the "Kozak sequence" included upstream of the antigen coding region?

(A) It acts as the binding site for the eukaryotic ribosome to ensure efficient translation initiation
(B) It initiates replication of the plasmid in E. coli
(C) It terminates transcription
(D) It provides resistance to kanamycin

2023

**254.** When expressing a viral glycoprotein in transgenic plants, targeting the protein to which cellular organelle often results in the highest stable accumulation?

(A) Endoplasmic reticulum
(B) Nucleus
(C) Mitochondria
(D) Lysosome

2006

**255.** Determine the correct order of the molecular cascade following administration of an mRNA vaccine encapsulated in a lipid nanoparticle (LNP).

(A) Endocytosis of the LNP, endosomal escape of mRNA into the cytosol, translation of mRNA by host ribosomes into protein, presentation of antigen on MHC molecules
(B) Translation of mRNA, endocytosis of LNP, endosomal escape, presentation of antigen
(C) Endocytosis of LNP, presentation of antigen, endosomal escape, translation of mRNA
(D) Endosomal escape, endocytosis of LNP, translation of mRNA, presentation of antigen

NODIA

2009

**256.** Identify the INCORRECT statement regarding synthetic peptide vaccines.

(A) They are highly stable and easily synthesized in pure form
(B) They represent the safest class of vaccines as they contain no infectious material
(C) They exhibit exceptional immunogenicity as naked, standalone molecules
(D) They require precise knowledge of the pathogen's specific epitopes

2002

**257.** A major advantage of using the DIVA strategy in the eradication of a disease is that it enables:

(A) Widespread vaccination while preserving the "disease-free" international trade status of a

(D) A is false but R is true.

2012

**273.** The primary immunological rationale for administering "booster" vaccinations is to:
(A) Induce a secondary immune response characterized by rapid production of high-affinity IgG and expansion of memory pools
(B) Force the immune system to un-learn the previous antigen
(C) Trigger an acute inflammatory response to clear residual primary vaccine
(D) Allow the animal to excrete the initial adjuvant

2022

**274.** Inactivated whole-agent vaccines are routinely created using beta-propiolactone (BPL) instead of formaldehyde for specific viral targets because BPL:
(A) Effectively inactivates nucleic acids with minimal alteration of structural surface epitopes
(B) Destroys proteins faster than formaldehyde
(C) Is intensely green, providing a visual marker of inactivation
(D) Stimulates viral replication prior to killing

2017

**275.** Which subset of antigen-presenting cells is uniquely capable of activating naive T-cells during the primary immune response?
(A) Dendritic cells
(B) Macrophages
(C) B lymphocytes
(D) Neutrophils

2009

**276.** Consider the following statements regarding eukaryotic expression systems utilizing Baculovirus:
I. They infect insect cells.
II. They are driven by the exceedingly strong polyhedrin promoter.
III. They are entirely incapable of folding complex mammalian glycoproteins.
IV. They frequently yield very high levels of recombinant protein.
Which of the above statement(s) is/are correct?
(A) I, II, and IV
(B) I, III, and IV
(C) II, III, and IV
(D) I and II

2012

**277.** The successful generation of a rationally attenuated virus via reverse genetics inherently requires starting from which type of molecule?
(A) An infectious complementary DNA (cDNA) clone of the viral genome
(B) A naturally occurring live virus
(C) A purified viral structural protein
(D) A monoclonal antibody against the virus

2009

**278.** Plasmid DNA vaccines frequently contain unmethylated CpG motifs. These motifs structurally mimic which type of naturally occurring DNA?
(A) Bacterial and viral DNA
(B) Mammalian nuclear DNA
(C) Plant mitochondrial DNA
(D) Synthetic plastic DNA

2013

**279.** Which significant obstacle prevents the widespread commercialization of edible plant-based vaccines?
(A) Regulatory challenges regarding dosage standardization and environmental contamination concerns
(B) The absolute inability of plants to synthesize proteins
(C) The plants immediately dying upon transformation
(D) The high cost of sunlight and water compared to bioreactors

2005

**280.** Identify the INCORRECT statement regarding the Jerne immune network theory.
(A) It postulates that the immune system is a network of interacting antibodies and anti-idiotypic antibodies
(B) Ab1 possesses idiotopes recognized by Ab2
(C) Ab2 acts purely as an immunosuppressive agent and never stimulates immunity
(D) Ab2 beta resembles the three-dimensional structure of the nominal antigen

2017

**281.** The process of B-cell epitope mapping for synthetic peptide vaccines relies on identifying sequences that are:
(A) Hydrophilic, highly mobile, and located on the surface of the native protein
(B) Highly rigid and entirely buried in the hydrophobic core
(C) Composed entirely of non-polar amino acids
(D) Identical to host protein sequences

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after inoculation; in a fed-batch culture, specific nutrients are added to prolong the growth phase

(B) A batch culture operates under strict vacuum; fed-batch operates under pressure

(C) Batch cultures are used for bacteria; fed-batch strictly for viruses

(D) Fed-batch continuously removes cells; batch does not

2001

**266.** Which physical property of an antigen is fundamentally exploited during ion-exchange chromatography in downstream purification?

(A) Net surface charge at a specific pH

(B) Molecular weight

(C) Hydrophobicity

(D) Specific affinity for a biological ligand

2013

**267.** Identify the INCORRECT statement regarding the use of preservatives in vaccine formulations.

(A) They are essential in multi-dose vials to prevent microbial growth after punctures

(B) Thimerosal is a mercury-containing compound historically used as a preservative

(C) Preservatives are heavily utilized in lyophilized single-dose live vaccines to keep the virus alive

(D) High concentrations of preservatives can negatively impact the potency of live-attenuated vaccines

2007

**268.** Match the regulatory term in List I with its definition in List II:

List I: (Regulatory Term)

P. Good Manufacturing Practice (GMP)

Q. Target Animal Safety

R. Batch Release

S. Pharmacopeia

List II: (Definition)

1. The process of verifying that a specific lot of vaccine meets all standard criteria before sale
2. An official publication containing rules and standardized procedures for manufacturing medicinal drugs
3. Evaluating the occurrence of adverse effects in the intended host species
4. A system for ensuring that products are consistently produced and controlled according to quality standards

(A) P-4, Q-3, R-1, S-2

(B) P-2, Q-1, R-4, S-3

(C) P-4, Q-1, R-3, S-2

(D) P-3, Q-2, R-1, S-4

NODIA

2025

**269.** Determine the correct sequence of phases in the lifecycle of a clinical trial for a new veterinary vaccine.

(A) Laboratory immunogenicity and safety, dose-determination, large-scale field efficacy, post-marketing surveillance

(B) Large-scale field efficacy, laboratory immunogenicity, post-marketing surveillance, dose-determination

(C) Post-marketing surveillance, large-scale field efficacy, dose-determination, laboratory immunogenicity

(D) Dose-determination, post-marketing surveillance, laboratory immunogenicity, large-scale field efficacy

2023

**270.** In the context of post-production testing, a "purity test" specifically verifies what?

(A) That the final product is free from contaminating adventitious microorganisms, chemicals, or cellular debris

(B) That the vaccine induces 100% protection

(C) That the vaccine contains exactly 1 ml of fluid

(D) That the adjuvant is purely synthetic

2016

**271.** An ideal veterinary vaccine must balance safety and efficacy. Which vaccine type generally offers the highest safety but the lowest intrinsic immunogenicity?

(A) Purified recombinant subunit proteins

(B) Live-attenuated mutants

(C) Replication-competent viral vectors

(D) Classically attenuated live strains

2024

**272.** Read the following statements and choose the correct option:

**Assertion (A):** Oral vaccines are uniquely suited for inducing secretory IgA at mucosal surfaces.

**Reason (R):** The gut-associated lymphoid tissue (GALT) contains specialized M-cells and dendritic cells that sample antigens and promote IgA class switching in B cells.

(A) Both A and R are true and R is the correct explanation of A.

(B) Both A and R are true but R is not the correct explanation of A.

(C) A is true but R is false.

region
(B) The virus to be completely eliminated from the environment within 24 hours
(C) The use of wild-type virus as the vaccine
(D) Farmers to stop testing animals altogether

2013
**258.** Reverse vaccinology screening specifically filters out candidate genes that share significant homology with the host genome. Why is this critical?
(A) To prevent the induction of autoimmune responses against host tissues
(B) To ensure the protein can be sequenced easily
(C) To maximize the size of the resulting vaccine
(D) To guarantee the protein is highly soluble

2002
**259.** Cryo-electron microscopy (Cryo-EM) revolutionized structural vaccinology because it allows researchers to:
(A) Determine the high-resolution, three-dimensional structure of complex viral glycoproteins in their native state without crystallization
(B) Boil the antigen and observe its primary sequence
(C) Measure the exact molecular weight of a DNA plasmid
(D) Quantify the amount of adjuvant in a vial

2022
**260.** The application of transcriptomics in Systems Vaccinology allows for the identification of early "molecular signatures." What is the value of these signatures?
(A) They can accurately predict the magnitude of the antibody response months later
(B) They replace the need for an adjuvant
(C) They determine the expiration date of the vaccine
(D) They sterilize the injection site

2008
**261.** Read the following statements and choose the correct option:
**Assertion (A):** Adversomics aims to reduce vaccine-related adverse events by abandoning mass vaccination protocols entirely.
**Reason (R):** Adversomics utilizes immunogenetic profiling to identify susceptible subpopulations and design safer formulations or personalized protocols.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2002
**262.** Aluminum-based adjuvants operate primarily through which distinct mechanism at the cellular level?
(A) Inducing lysosomal destabilization upon phagocytosis, which triggers the NLRP3 inflammasome to secrete IL-1beta
(B) Direct binding to and activation of TLR4
(C) Inserting directly into the host nucleus
(D) Digesting host cell membranes

2003
**263.** When cytokines are used as vaccine enhancers, why is delivering the cytokine gene via a plasmid or viral vector often preferred over injecting the recombinant cytokine protein?
(A) Cytokine genes ensure sustained, localized expression, overcoming the extremely short halF-life of injected proteins
(B) Cytokine proteins are strictly insoluble in water
(C) Injected cytokine proteins instantly cause fatal anaphylaxis in all cases
(D) Plasmids are 100% efficient at transfecting every cell in the body

2023
**264.** Consider the following statements regarding ISCOMs (Immune Stimulating Complexes):
I. They are cage-like particles typically 40 nm in diameter.
II. They incorporate saponins, cholesterol, and phospholipids alongside the antigen.
III. They are highly efficient at delivering antigens into the cytosol for MHC Class I presentation.
IV. They are utilized exclusively for bacterial toxoid vaccines.
Which of the above statement(s) is/are correct?
(A) I, II, and III
(B) I, III, and IV
(C) II, III, and IV
(D) I and II

2003
**265.** What is the fundamental operational difference between a batch bioreactor culture and a fed-batch bioreactor culture in vaccine manufacturing?
(A) In a batch culture, no nutrients are added

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2015

**282.** The DIVA strategy for infectious bovine rhinotracheitis typically relies on a vaccine lacking the gE glycoprotein. For the strategy to work, the gE protein in wild-type strains must be:
(A) Non-essential for in vitro replication and highly immunogenic
(B) Essential for in vitro replication but non-essential in vivo
(C) The sole binding site for the host cell receptor
(D) Structurally identical to a host cell protein

2022

**283.** During reverse vaccinology, after in silico identification of putative antigens, what is the mandatory next step to validate their potential?
(A) Cloning, expressing the recombinant proteins, and screening them for immunogenicity and protective efficacy
(B) Releasing the sequence immediately to a clinical trial
(C) Deleting the genes from the host animal
(D) Changing the genetic code of the pathogen to a plant code

2002

**284.** Structural vaccinology aims to optimize antigens. If an antigen possesses a highly flexible, non-neutralizing loop, a structural approach might:
(A) Delete or mask this loop to redirect the response toward conserved, sub-dominant neutralizing epitopes
(B) Enlarge the loop tenfold
(C) Unfold the entire protein to a linear chain
(D) Attach a massive bacterial toxin to the loop

2009

**285.** In Vaccinomics, high-dimensional flow cytometry is utilized to evaluate:
(A) The precise subsets and activation states of the diverse cellular immune compartments responding to the vaccine
(B) The physical color of the vaccine fluid
(C) The nucleotide sequence of the antigen
(D) The rate of lyophilization

2023

**286.** What is the fundamental goal of identifying an adversomic biomarker prior to a vaccination campaign?
(A) To screen out and exclude genetically susceptible individuals from receiving a specific vaccine formulation
(B) To increase the overall cost of the vaccine
(C) To identify which animals have the best coat color
(D) To ensure the vaccine induces disease

NODIA

2002

**287.** Consider the following statements regarding Toll-Like Receptor (TLR) agonists as adjuvants:
I. Monophosphoryl lipid A (MPL) is a detoxified derivative of LPS that acts as a TLR4 agonist.
II. TLR agonists function by bridging the innate and adaptive immune systems.
III. They operate entirely independently of antigen-presenting cells.
IV. TLR agonists generally skew the immune response toward a Th1 profile.
Which of the above statement(s) is/are correct?
(A) I, II, and IV
(B) II, III, and IV
(C) I and III
(D) I, II, and III

2001

**288.** Match the cytokine in List I with its targeted immunological effect in List II:
List I: (Cytokine)
P. IL-12
Q. IL-4
R. GM-CSF
S. IL-15
List II: (Immunological Effect)
1. Promotes the maintenance and survival of long-lived memory CD8+ T cells
2. Drives Th1 differentiation and cellular immunity
3. Enhances dendritic cell recruitment, maturation, and antigen presentation
4. Drives Th2 differentiation and robust humoral responses
(A) P-2, Q-4, R-3, S-1
(B) P-1, Q-2, R-4, S-3
(C) P-4, Q-3, R-1, S-2
(D) P-3, Q-1, R-4, S-2

2017

**289.** When designing a PLGA polymeric microsphere vaccine, adjusting the ratio of lactide to glycolide polymers strictly controls what crucial delivery parameter?
(A) The rate of polymer degradation and the subsequent kinetics of antigen release
(B) The exact amino acid sequence of the encapsulated antigen
(C) The replication rate of the encapsulated virus
(D) The color of the microspheres

2008

**290.** The integration of nanotechnology into vaccinology frequently employs "nanogels." What unique physicochemical property makes nanogels ideal for mucosal delivery?
(A) They possess mucoadhesive properties and can respond to physiological triggers like pH or temperature
(B) They are solid as rock, preventing any degradation
(C) They are highly hydrophobic and repel water
(D) They consist entirely of heavy metals

2001

**291.** In downstream processing of large-scale recombinant vaccines, which technique removes large cellular debris while allowing the soluble protein product to pass into the permeate?
(A) Tangential Flow Filtration
(B) Reverse osmosis
(C) Lyophilization
(D) Isoelectric focusing

2023

**292.** Which of the following describes the "cold chain" in vaccine logistics?
(A) The sequence of frozen bioreactors used during upstream processing
(B) The system of transporting and storing vaccines within a recommended temperature range from manufacture to administration
(C) The process of cooling animals prior to vaccination to reduce fever
(D) The use of liquid nitrogen to instantly kill pathogens during production

2014

**293.** According to pharmacopeial guidelines, what does the "potency" test of a veterinary vaccine explicitly evaluate?
(A) The quantitative measure of the capacity of the product to achieve its intended immunologic effect
(B) The specific gravity of the vial
(C) The color of the vaccine cake
(D) The sterility of the rubber stopper

2008

**294.** Determine the correct sequence for completing the development of a veterinary vaccine.
(A) Target animal safety studies, dose titration, laboratory challenge efficacy, field efficacy trials
(B) Field efficacy trials, dose titration, target animal safety studies, laboratory challenge efficacy
(C) Dose titration, field efficacy trials, laboratory challenge efficacy, target animal safety studies
(D) Laboratory challenge efficacy, target animal safety studies, dose titration, field efficacy trials

2017

**295.** Read the following statements and choose the correct option:
**Assertion (A):** The limulus amebocyte lysate (LAL) test is an essential quality control assay for recombinant vaccines produced in E. coli.
**Reason (R):** E. coli is a Gram-negative bacterium, and the LAL test is highly sensitive for detecting residual lipopolysaccharides in the final product.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2019

**296.** In mammalian cellular immune responses, what is the required costimulatory signal on an antigen-presenting cell that binds to CD28 on a naive T cell to enable full activation?
(A) CD80/86 (B7-1/B7-2)
(B) CD4
(C) CD8
(D) MHC Class II

2001

**297.** Consider the following statements regarding killed traditional vaccines:
I. They inherently require the use of chemical or physical inactivation methods.
II. They represent zero risk of shedding live pathogenic virus into the environment.
III. They generally stimulate powerful cytotoxic CD8+ T-cell responses natively.
IV. They often require a prime-boost regimen to maintain protective immunity.
Which of the above statement(s) is/are correct?
(A) I, II, and IV
(B) II, III, and IV
(C) I and III
(D) I, II, and III

2007

**298.** Poxvirus vector platforms possess an inherently safe profile for use in diverse host species because:
(A) They have lost the ability to replicate efficiently in most mammalian cells,

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undergoing an abortive infection
(B) They integrate their DNA seamlessly into the host genome
(C) They lack an envelope completely
(D) They do not infect any known cell type

2004

**299.** In the creation of marker vaccines via targeted gene deletion, deleting the thymidine kinase (TK) gene specifically achieves what?
(A) It attenuates the virus by reducing its ability to replicate in non-dividing cells like neurons
(B) It serves strictly as the marker for the companion diagnostic test
(C) It increases the virulence of the strain
(D) It makes the virus invisible to the immune system

2011

**300.** The ultimate objective of Systems Vaccinology and multi-omics profiling is closely aligned with the concept of "correlates of protection." This fundamentally means finding a biomarker that:
(A) Is a surrogate metric associated with protection from disease
(B) Is a geographical identifier proving regional animal origin
(C) Is a biochemical value for the molecular weight of adjuvants
(D) Is a visual standard for the specific color of vaccine vials

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2006

**241.** A nanogel used for targeted mucosal delivery relies on its ability to respond to specific environmental stimuli. Which is a common stimulus triggering antigen release in the gut?
(A) Shifts in pH and the presence of specific degrading enzymes
(B) Extreme cold
(C) Complete darkness
(D) High external pressure

2024

**242.** In large-scale suspension cell cultures for viral vaccine production, the switch from batch mode to continuous perfusion mode offers what primary advantage?
(A) Constant removal of toxic metabolic byproducts and continuous supply of nutrients, leading to vastly higher cell densities
(B) Absolute elimination of the need for oxygen
(C) Immediate sterilization of the product
(D) Use of pure water without media

2005

**243.** Identify the INCORRECT statement regarding the lyophilization of vaccines.
(A) It transforms a liquid vaccine into a stable dry powder
(B) The process subjects the antigen to both freezing and desiccation stress
(C) Freezing rates do not affect the viability of the live virus or the protein structure
(D) It requires specific lyoprotectants to maintain the physical structure of the biological material

2022

**244.** According to regulatory frameworks, what is the function of the "Master Cell Seed" in vaccine production?
(A) To provide a fully characterized, standardized, and pathogen-free cell line for all future production
(B) To randomly generate new cell lines for each batch
(C) To act as the animal model for challenge studies
(D) To serve as the adjuvant in the final formulation

2009

**245.** Read the following statements and choose the correct option:
**Assertion (A):** In pre-clinical trials, finding the precise minimum immunizing dose is critical.
**Reason (R):** Excessive antigen payloads can lead to immune tolerance, increased production costs, and higher risks of adverse site reactions.
(A) Both A and R are true and R is the correct explanation of A.
(B) Both A and R are true but R is not the correct explanation of A.
(C) A is true but R is false.
(D) A is false but R is true.

2002

**246.** In post-production quality control, a test for "residual moisture" is mandatory for which type of vaccine?
(A) Lyophilized live vaccines
(B) Liquid inactivated bacterins
(C) Oil-emulsion vaccines
(D) Naked DNA plasmid solutions

2005

**247.** The primary reason for administering a tetanus toxoid vaccine for long-term prophylaxis is:
(A) Toxoids stimulate active immunity and the formation of memory B cells for long-term protection
(B) Toxoids provide an immediate neutralizing effect within minutes
(C) Antitoxins cause immediate fatal anaphylaxis in all hosts
(D) Toxoids are administered orally

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2003

**248.** The live-attenuated Brucella abortus Strain 19 vaccine occasionally caused disease in humans. This highlights which fundamental disadvantage of classical live-attenuated vaccines?
(A) They possess an inherent risk of residual virulence or reversion, particularly in non-target or immunocompromised hosts
(B) They completely fail to stimulate cell-mediated immunity
(C) They are purely synthetic and lack biological relevance
(D) They cannot be lyophilized

2006

**249.** Consider the following statements regarding the host mucosal immune response:
I. Secretory IgA is a dimer linked by a J chain and a secretory component.
II. The secretory component protects sIgA from proteolytic cleavage in mucosal environments.
III. Systemic intramuscular vaccines are universally superior at inducing mucosal

**9.** (D) The internal image of the Ab2 variable region
The Jerne immune network theory describes how anti-idiotypic antibodies can function as vaccine components. Specifically, certain secondary antibodies possess variable regions that represent an internal image of the original pathogen's epitopes. When administered, these molecules mimic the structure of the antigen, tricking the immune system into producing protective antibodies against the pathogen without using the actual infectious agent.

**10.** (A) Lack of T-helper cell epitopes in the peptide
Synthetic peptide vaccines often consist of small sequences that represent specific B-cell epitopes. These short peptides frequently lack the necessary T-helper cell epitopes required to initiate a full adaptive immune response. By chemically coupling the peptide to a large carrier protein like Keyhole Limpet Hemocyanin, the immune system receives the essential signals needed to activate B cells.

**11.** (B) Absence of a specific antigen found in wild-type strains
A successful Differentiating Infected from Vaccinated Animals strategy depends on the absence of a specific immunogenic protein in the vaccine strain. This missing component serves as a negative marker. Diagnostic tests then look for antibodies against this specific protein; their presence indicates natural infection by the wild-type pathogen, while their absence confirms that the animal has only been vaccinated.

**12.** (B) In silico bioinformatic analysis of the pathogen genome
Reverse vaccinology represents a paradigm shift by utilizing bioinformatic tools to analyze the entire genome of a pathogen before any laboratory cultivation occurs. This in silico approach identifies potential surface-exposed proteins and secreted antigens that are most likely to be protective. This method is significantly faster and more comprehensive than traditional biochemical purification or culture-based discovery techniques used previously.

**13.** (A) X-ray crystallography
Structural vaccinology relies on high-resolution imaging techniques to map the precise three-dimensional configuration of viral and bacterial antigens. X-ray crystallography is central to this field, providing the atomic-level detail necessary for rational design. This information allows scientists to engineer stable versions of proteins that expose neutralizing epitopes, which is particularly useful for designing vaccines against highly complex or mutable pathogens.

**14.** (C) Prediction of host immune responses and vaccine efficacy
Systems vaccinology employs transcriptomic profiling to measure the global gene expression changes within the host following immunization. By analyzing thousands of RNA transcripts simultaneously, researchers can identify early molecular signatures that correlate with the development of protective immunity. This comprehensive approach allows for the prediction of vaccine efficacy and a better understanding of the underlying mechanisms of protection.

**15.** (D) Genetic basis of severe adverse reactions to vaccines
Adversomics is an emerging field that focuses on the immunogenetic profiling of populations to understand the biological basis of severe adverse reactions to vaccines. By identifying specific genetic markers or variations within a host, researchers can predict which individuals are at higher risk for complications. This knowledge is crucial for developing safer vaccine formulations and personalized immunization strategies for susceptible groups.

**16.** (A) P-2, Q-4, R-1, S-3
Different adjuvants enhance the immune response through distinct mechanisms. Aluminum salts create a depot effect and activate the inflammasome, while Freund's Complete Adjuvant uses mycobacterial components to drive Th1 responses. CpG oligodeoxynucleotides act as TLR9 agonists, and immune stimulating complexes deliver antigens into the cytosol for MHC Class I presentation. Matching these components is essential for tailoring the desired response.

**17.** (B) Interleukin-12
The polarization of the T-helper cell response is critical for effective immunity against various pathogens. Interleukin-12 is a key cytokine produced by dendritic cells that promotes the differentiation of naive T cells into the Th1 subset. Incorporating this cytokine into vaccine formulations helps drive cell-mediated immune

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**35.** (A) High hydrophilicity and surface exposure
The identification of B-cell epitopes is a fundamental step in designing synthetic peptide vaccines. These epitopes represent the specific parts of an antigen that are recognized by antibodies. Successful candidates are typically found in regions that exhibit high hydrophilicity and are located on the surface of the protein. This exposure ensures they are accessible to B-cell receptors and circulating antibodies.

**36.** (C) Competitive ELISA detecting antibodies against the deleted antigen
A Differentiating Infected from Vaccinated Animals strategy requires a specific diagnostic test to be used alongside a marker vaccine. When the vaccine is created via a targeted gene deletion, the most common companion assay is a competitive ELISA. This test specifically detects the presence of antibodies against the deleted protein, which would only be found in animals naturally infected with the virus.

**37.** (A) Inability to identify non-protein antigens like polysaccharides
While reverse vaccinology is a powerful tool for identifying protein antigens from genomic data, it has significant limitations. The primary drawback is its inability to identify non-protein antigens, such as the polysaccharide capsules found on many bacteria. Since these structures are not directly encoded by a single gene but are synthesized by complex pathways, they cannot be discovered through simple genomic screening.

**38.** (D) Viral envelope fusion glycoproteins
Structural vaccinology has been instrumental in modernizing vaccine design by focusing on the precise atomic structure of viral components. A major application of this field is the stabilization of viral envelope fusion glycoproteins in their pre-fusion state. Engineering these antigens to remain in this specific conformation prevents them from triggering prematurely, thereby exposing the most important neutralizing epitopes to the immune system.

**39.** (B) The individual and population-level variability in vaccine responses
Vaccinomics utilizes large-scale multi-omics data to study the interactions between the host immune system and vaccines. One of its primary objectives is to address the significant individual and population-level variability in vaccine responses. By understanding how genetic and environmental factors influence immunity, researchers can develop more effective vaccines and strategies that account for the diverse biological backgrounds of target populations.

**40.** (A) Identify the desired immune response profile, evaluate compatibility with the antigen, conduct safety and toxicity testing, assess long-term stability
The selection of an appropriate adjuvant for a vaccine formulation follows a logical sequence of engineering and biological considerations. First, the desired immune response profile must be identified. Next, the adjuvant's compatibility with the specific antigen is evaluated. This is followed by rigorous safety and toxicity testing, and finally, the long-term stability of the combined formulation is assessed to ensure efficacy.

**41.** (D) Cytotoxic T lymphocyte (CTL) responses and Th1 immunity
Saponin-based adjuvants, such as Quil A, are known for their potent immunostimulatory properties in veterinary vaccines. They are particularly effective at inducing strong cell-mediated immune responses, including the activation of cytotoxic T lymphocytes and Th1-type immunity. This makes them highly valuable for vaccines targeting intracellular pathogens, where a robust cellular defense is required alongside the production of specific neutralizing antibodies.

**42.** (A) Recruitment of antigen-presenting cells to the site of immunization
Chemokines are increasingly used as biological vaccine enhancers to improve the overall immune response. These signaling proteins function primarily by promoting the recruitment of essential antigen-presenting cells, such as dendritic cells, to the site of immunization. By increasing the local concentration of these cells, the vaccine ensures more efficient processing and presentation of the antigen, leading to a stronger adaptive response.

**43.** (B) Proteolytic and enzymatic degradation
Liposomal encapsulation provides a protective environment for vaccine antigens after administration into the body. This delivery system is particularly effective at shielding the antigen from various in vivo processes that

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from its mother without its own immune system being actively involved. In cattle, this occurs through the ingestion and absorption of maternal colostrum shortly after birth. This process provides immediate, though temporary, protection against environmental pathogens during the critical early weeks of life while the calf's immune system develops.

**27.** (B) Nucleic acids
Chemical inactivation is a standard method for preparing killed vaccines, and beta-propiolactone is a commonly used agent for this purpose. It primarily targets and modifies the nucleic acids of the pathogen, effectively preventing genomic replication. A major advantage of this chemical is that it preserves the structural integrity of the surface proteins, ensuring that the resulting vaccine remains highly immunogenic.

**28.** (B) Cytotoxic CD8+ T lymphocytes
Once a viral infection is established within host cells, the immune system must identify and eliminate the infected cells to stop replication. Cytotoxic CD8+ T lymphocytes are the primary effectors for this task. Following vaccination, these cells are primed to recognize specific viral peptides presented on the cell surface, allowing them to induce apoptosis in infected cells and clear the infection.

**29.** (A) Capability for complex post-translational modifications
Eukaryotic expression systems, such as those using baculovirus and insect cells, offer a distinct advantage over prokaryotic systems like Escherichia coli. These higher organisms possess the complex cellular machinery required for essential post-translational modifications, including proper folding and glycosylation. This ensures that the recombinant subunit antigens closely mimic the native structure of the pathogen, which is vital for inducing immunity.

**30.** (D) II
Salmonella vectors are highly effective because they are naturally adapted to mucosal surfaces and can be engineered to deliver DNA vaccines or antigens. They are usually attenuated by deleting metabolic genes like aroA to ensure safety. However, these vectors are typically administered orally to target the gut-associated lymphoid tissue, rather than being given through intramuscular injection, which is not the standard.

**31.** (D) Essential metabolic intermediates (e.g., purines)
Rational attenuation of bacterial pathogens often involves the targeted deletion of genes required for synthesizing essential metabolic intermediates. For example, removing the ability to produce purines or aromatic amino acids prevents the bacteria from replicating effectively within the host's nutrient-limited tissues. This results in a safe vaccine strain that can still stimulate the immune system without causing a full infection.

**32.** (C) To enable amplification and production of the plasmid in E. coli prior to formulation
In the context of DNA vaccine technology, the bacterial origin of replication is a critical structural element within the plasmid. This component is not used by the vaccinated animal's cells. Instead, its primary function is to allow the plasmid to be efficiently amplified and produced in large quantities within Escherichia coli cultures during the manufacturing process prior to vaccine formulation.

**33.** (B) High levels of antigen accumulation without gene silencing
Chloroplast engineering in plant-based expression systems offers several major advantages for vaccine production. One of the most significant features is the ability to achieve exceptionally high levels of antigen accumulation within the plant tissue. Unlike nuclear transformation, chloroplast-expressed genes are not subject to gene silencing, ensuring consistent and robust production of the desired protein for use in the final vaccine.

**34.** (D) The primary antibody raised against the original target antigen
Within the framework of the Jerne immune network theory, different antibodies are categorized based on their interactions. The term Ab1 refers to the primary antibody that is initially produced in response to the original target antigen of the pathogen. Subsequent antibodies in the network, such as Ab2, are then raised against the variable regions of this primary Ab1 molecule to create mimicry.

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**88.** (D) Trigger-induced conformational changes that hide neutralizing epitopes
Structural vaccinology uses computational design to optimize antigens, such as locking viral fusion glycoproteins into a pre-fusion state. This engineering is critical because it prevents the antigen from undergoing trigger-induced conformational changes after administration. By maintaining the pre-fusion structure, the vaccine ensures that the most important neutralizing epitopes remain exposed to the immune system, leading to a more potent response.

**89.** (A) Metabolomics
Metabolomics is the multi-omics approach that focuses on analyzing the complete set of small-molecule metabolites within an organism. In the context of vaccinology, this technology is used to assess the animal's physiological and metabolic response to a vaccine. By monitoring changes in these molecules, researchers can gain insights into the vaccine's safety, efficacy, and its overall impact on host health.

**90.** (D) I, III, and IV
Adversomics is a critical field that utilizes genome-wide association studies to identify genetic predispositions to vaccine-associated adverse events. This approach aims to design safer vaccines for genetically susceptible populations by understanding the host's unique biological background. While it provides essential safety information, it does not replace standard Phase I trials but rather complements them by offering a deeper understanding of individual risks.

**91.** (D) Severe local tissue site reactions and granulomas
Water-in-oil emulsions, such as Freund's Incomplete Adjuvant, are powerful tools for creating a long-lasting depot effect at the injection site. However, their use is frequently restricted in food-producing animals. This is primarily because they can cause severe local tissue reactions and the formation of granulomas. These reactions are undesirable as they can affect animal welfare and damage meat quality at slaughter.

**92.** (D) Long-lived memory CD8+ T cells
Incorporating Interleukin-15 into a viral vaccine formulation is a strategic approach to improving long-term immunity. This cytokine plays a vital role in the immune system by enhancing the development and survival of long-lived memory CD8+ T cells. By promoting these specific cells, the vaccine ensures that the animal maintains a robust defense that can be rapidly reactivated upon future exposure.

**93.** (A) Antigen, cholesterol/phospholipid, and saponin (Quil A)
Immune Stimulating Complexes are advanced particulate delivery systems that form unique cage-like structures. The formation of these complexes fundamentally requires three specific components: the target antigen, cholesterol or phospholipids, and a saponin derivative such as Quil A. This combination is essential for creating the organized structure that effectively delivers antigens to immune cells and stimulates both humoral and cellular responses.

**94.** (B) Highly branched, tree-like, symmetrically spherical macromolecules
In the field of nanotechnology-based vaccinology, dendrimers are a unique class of delivery vehicles. These molecules are characterized by their highly branched, tree-like, and symmetrically spherical structural organization. Their precise macromolecular design allows for the attachment of multiple antigens and adjuvants to their surface, making them versatile platforms for developing highly controlled and potent vaccine formulations for various applications.

**95.** (A) Oxygen
During the scale-up of bioreactor operations for vaccine production, engineers must manage several critical parameters. The mass transfer of oxygen is usually the most limiting factor because of its low solubility in liquid culture media. Ensuring an adequate supply of oxygen to rapidly growing cells requires precise control over agitation and aeration to prevent nutrient deprivation and maintain high productivity.

**96.** (C) Maintaining optimal pH for antigen stability
Buffers such as phosphate or histidine are essential components of many vaccine formulations. Their primary role is to maintain the optimal pH level required for the chemical and physical stability of the antigen. By preventing significant shifts in acidity or alkalinity during storage and transport, buffers help ensure that the vaccine remains potent and effective until it is administered to the animal.

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could reduce its efficacy, such as proteolytic and enzymatic degradation. By keeping the antigen intact until it is taken up by target cells, liposomes ensure that the immune system receives the full dose.

**44.** (B) They possess mucoadhesive properties and respond to environmental stimuli (e.g., pH)
Nanogels are advanced particulate delivery systems that offer unique biochemical advantages for targeted mucosal vaccination. These structures possess inherent mucoadhesive properties that allow them to remain in contact with mucosal surfaces longer. Furthermore, they can be engineered to respond to specific environmental stimuli, such as changes in pH, allowing for the precise release of the vaccine antigen at the desired location.

**45.** (A) By adjusting the sparging rate of oxygen/air and impeller agitation speed
In industrial vaccine manufacturing, maintaining the dissolved oxygen concentration within the bioreactor is critical for ensuring optimal cell growth and productivity. This parameter is typically regulated through a combination of engineering controls. Operators adjust the sparging rate of air or pure oxygen into the media and modify the impeller agitation speed to facilitate efficient gas transfer throughout the entire culture volume.

**46.** (A) P-2, Q-1, R-4, S-3
Vaccine formulations include various excipients with specific roles. Thimerosal serves as an antimicrobial preservative in multi-dose vials, while Polysorbate 80 acts as a surfactant to prevent protein aggregation. Sucrose is utilized as a lyoprotectant to stabilize structures during the freeze-drying process. Finally, aluminum hydroxide is a standard adjuvant used to enhance the overall immunogenicity of the final vaccine product.

**47.** (D) Mycoplasma and adventitious viruses
National regulatory authorities require stringent purity testing for every batch of a veterinary vaccine before it can be released to the market. The primary focus of these tests is to confirm the total absence of harmful contaminants. This includes screening for mycoplasma and various adventitious viruses that may have been introduced during the manufacturing process, ensuring the final product is safe.

**48.** (C) The lowest antigen payload required to induce a statistically significant protective immune response
During the pre-clinical phase of vaccine development, researchers must perform dose-determination studies to find the optimal antigen level. The minimum immunizing dose is specifically defined as the lowest antigen payload required to induce a statistically significant protective immune response in the target species. Identifying this value is crucial for balancing vaccine efficacy with manufacturing costs and minimizing the risk of adverse reactions.

**49.** (B) Quantification of specific antigen mass using ELISA
The field of vaccine quality control is moving away from animal-based challenge models toward more reproducible in vitro metrics. For killed bacterial vaccines, a standard in vitro potency test involves the quantification of specific antigen mass using techniques like ELISA. This approach provides a precise measurement of the active ingredients in each batch, ensuring consistency and safety while following modern ethical guidelines.

**50.** (A) Both A and R are true and R is the correct explanation of A.
Killed viral vaccines generally require the administration of multiple booster doses to establish and maintain a level of protective immunity. This is because inactivated antigens do not replicate within the host, leading to a significantly shorter duration of antigenic stimulation compared to live vaccines. Consequently, frequent reinforcements are necessary to sustain the memory response and ensure continued protection against the pathogen.

**51.** (B) Shorter lag phase, faster rise, and higher plateau of IgG
The secondary immune response occurs when the immune system encounters an antigen for a second time. This response is much more efficient than the primary one, characterized by a shorter lag phase and a faster rise in antibody levels. The resulting titers reach a higher plateau, with a predominance of high-affinity IgG antibodies and a robust involvement of memory T cells.

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**70.** (B) Affinity chromatography
During the downstream processing of large-scale recombinant vaccines, high levels of purity are required for the final product. Affinity chromatography is the technique predominantly used for this purpose because of its exceptional specificity. This method utilizes a unique biological ligand that binds only to the target antigen, allowing all other cellular proteins and contaminants to be washed away during purification.

**71.** (B) Microbial contamination after the vial is punctured
Preservatives are essential additives for multi-dose vaccine vials used in veterinary practice. Their primary role is to prevent microbial contamination that can occur each time the vial's rubber stopper is punctured by a needle. By inhibiting the growth of bacteria and fungi, preservatives ensure that the remaining doses in the vial stay safe and sterile for subsequent use in other animals.

**72.** (C) World Organisation for Animal Health (WOAH)
The World Organisation for Animal Health is the primary international body responsible for animal health standards. It establishes comprehensive diagnostic and vaccine guidelines for both terrestrial and aquatic diseases worldwide. By coordinating global efforts, the organization ensures that veterinary practices are standardized, which is vital for controlling outbreaks, protecting animal welfare, and facilitating the safe international trade of animals and products.

**73.** (B) Vaccine efficacy (VE)
In the context of a clinical field trial, the primary measure of a vaccine's performance is vaccine efficacy. This epidemiological parameter quantifies the reduction in the incidence of disease among the vaccinated group compared to the unvaccinated control group. A high efficacy value indicates that the vaccine is successful at protecting the target population from infection under real-world conditions and pressure.

**74.** (B) 2x or 10x the recommended dose
Regulatory guidelines for veterinary vaccines require rigorous in vivo safety testing before approval. One standard procedure involves administering a significant multiple of the normal field dose to the target species to monitor for adverse effects. Depending on the specific regulations, this typically involves testing either two times or ten times the recommended dose to ensure the product remains safe.

**75.** (A) Both A and R are true and R is the correct explanation of A.
DNA vaccines are innovative tools that can successfully induce both humoral and cell-mediated immunity without the use of live infectious agents. This is achieved because the plasmid DNA enters the host's own cells, where it drives the endogenous synthesis of the target antigen. These proteins are then processed and presented via both MHC Class I and Class II pathways, respectively.

**76.** (A) Unimmunized or vulnerable individuals in the community
The concept of herd immunity describes the indirect protection provided to a community when a large proportion of its members are immunized. This high level of collective immunity makes it difficult for a pathogen to spread, thereby protecting unimmunized or particularly vulnerable individuals. By reducing the overall transmission of the disease, widespread vaccination helps safeguard those who cannot be vaccinated.

**77.** (D) Treating the culture with formalin under controlled time and temperature
Producing an effective killed bacterial vaccine, or bacterin, requires a balance between complete inactivation and the preservation of essential surface antigens. The most common method involves treating the bacterial culture with formalin under carefully controlled conditions of time and temperature. This chemical cross-links the proteins, killing the pathogen while maintaining the structural integrity needed for the immune system to recognize it.

**78.** (D) CD8+ Cytotoxic T cells
The host immune response to intracellular parasites requires specialized cellular mechanisms to eliminate the infection. For pathogens like Theileria, which reside inside host cells, CD8+ cytotoxic T lymphocytes play the dominant role. These cells are specifically programmed to recognize parasite antigens presented on the surface of infected cells and then induce lysis or apoptosis to stop the parasite's lifecycle.

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**61.** (B) Non-structural proteins (NSPs) such as 3ABC
In the control of Foot-and-Mouth Disease, DIVA strategies are essential for distinguishing between vaccinated and naturally infected animals. Most modern FMD vaccines are highly purified and lack non-structural proteins. Therefore, diagnostic assays target these non-structural proteins, such as 3ABC. The presence of antibodies against these proteins indicates that the virus has actively replicated in the animal, confirming a natural infection.

**62.** (B) PRED-TMBB and SignalP
Reverse vaccinology relies on several specialized bioinformatic tools to screen pathogen genomes for potential vaccine candidates. Among these, PRED-TMBB and SignalP are indispensable for identifying critical structural features. These programs predict the presence of transmembrane domains and signal peptides, respectively, allowing researchers to pinpoint proteins that are likely to be surface-exposed or secreted, making them ideal targets for immunity.

**63.** (B) II and III
Structural vaccinology is a modern approach that utilizes advanced imaging technologies, such as cryo-electron microscopy, to resolve the precise configuration of proteins. The goal of this field is to computationally design and lock flexible viral proteins into their most immunogenic conformations. This ensures that the vaccine presents the immune system with the best possible targets for producing potent neutralizing antibodies.

**64.** (C) Proteomics
In the field of antigen discovery, proteomics is the specific technology used to measure and analyze the entire complement of proteins produced by an organism. By studying the proteome, researchers can identify which proteins are most abundant or located on the pathogen's surface during different stages of infection. This information is vital for selecting the most effective targets for vaccination.

**65.** (C) DLA
The Major Histocompatibility Complex is a critical genetic region that influences the immune response and the potential for adverse reactions. Every domestic species has its own specific nomenclature for this complex. In dogs, the MHC equivalent is known as Dog Leukocyte Antigen. Understanding the DLA genotype is essential in the field of adversomics to identify individual susceptibility to certain vaccines.

**66.** (C) TLR7/8
Modern vaccine adjuvants often include agonists that target specific Toll-like receptors to stimulate the innate immune system. For vaccines incorporating single-stranded RNA, the relevant sensors are TLR7 and TLR8. Activation of these receptors leads to the production of pro-inflammatory cytokines and promotes a robust adaptive immune response, making them effective tools for enhancing the potency of various vaccine formulations.

**67.** (C) Interferon-gamma
Designing vaccines against parasites often requires driving a specific immune response characterized by Th2 activity and IgE production. In this context, certain cytokines like Interleukin-12 and Interferon-gamma are least relevant because they are potent drivers of the opposing Th1 response. These molecules would actually suppress the desired Th2-mediated immunity, making them counterproductive for vaccines aimed at helminths or similar parasites.

**68.** (A) Primary emulsification (W1/O), secondary emulsification (W1/O/W2), solvent extraction/evaporation, particle collection/washing
The manufacture of PLGA microsphere vaccines using the double-emulsion solvent evaporation method follows a precise series of steps. It begins with the primary emulsification of the aqueous antigen into the organic polymer phase. This is followed by a secondary emulsification into a larger aqueous volume. Finally, the solvent is extracted or evaporated, allowing the particles to be collected and washed.

**69.** (B) Secretory IgA
Targeted mucosal delivery systems using nanotechnology aim to provide protection exactly where pathogens first enter the body. The primary objective of these methods is to induce high titers of secretory IgA at the mucosal surface. This specific antibody isotype is essential for neutralizing viruses and bacteria before they can cross the epithelial barrier, providing a powerful and localized defense.

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**52.** (C) Risk of disease in immunocompromised animals
A significant disadvantage of traditional live-attenuated vaccines in veterinary medicine is the risk they pose to certain vulnerable populations. Because the vaccine contains a living, though weakened, pathogen, it can occasionally cause actual disease in immunocompromised animals or those with underlying health issues. This necessitates careful screening and limits the use of such vaccines in herds with unknown or compromised health.

**53.** (B) Pathogen exclusion through mucosal neutralization
Secretory IgA is the most important immunoglobulin isotype involved in the defense of mucosal surfaces. Its primary function is pathogen exclusion, which is achieved by neutralizing viruses and bacteria directly at the site of entry. By preventing these pathogens from adhering to and colonizing the epithelial lining, secretory IgA provides a critical first line of defense against many common infectious diseases.

**54.** (B) I and IV
Eukaryotic expression systems, such as yeast, are highly effective for producing vaccine antigens because they can perform essential post-translational modifications like glycosylation. This capability ensures that the proteins are folded correctly and function as intended. However, these systems are generally more expensive and complex to scale up for large-scale production compared to simpler prokaryotic systems like Escherichia coli.

**55.** (A) It renders the vector replication-defective in normal host cells
Adenovirus vectors are widely used for vaccine delivery because of their efficiency in entering host cells. To improve safety, these vectors are typically engineered by deleting the $E_1$ region of the viral genome. This modification renders the vector replication-defective in normal host cells, ensuring that the virus can deliver the antigen DNA without causing an active, spreading infection in the animal.

**56.** (B) Mutant replicating at low temperatures but not at core body or febrile temperatures
Temperature-sensitive rationally attenuated mutants are a unique type of vaccine strain designed for mucosal administration. These organisms are engineered to replicate effectively at the cooler temperatures found in the upper respiratory tract but become inactive at the higher core body temperatures of the host. This restriction ensures localized immunity while preventing the pathogen from spreading internally and causing systemic disease.

**57.** (B) In vivo electroporation
The efficacy of naked plasmid DNA vaccines is often limited by poor cellular uptake following injection. To overcome this, physical methods such as in vivo electroporation are employed. This technique uses brief electrical pulses to create temporary pores in the host cell membranes, significantly enhancing the entry of the DNA. This results in much higher levels of antigen expression and immunity.

**58.** (A) Standardizing the exact antigen dose per consumed serving
Edible vaccines produced in transgenic plants face unique regulatory and physiological challenges that hinder their widespread use. One of the most significant hurdles is the difficulty in standardizing the exact dose of antigen per consumed serving. Factors such as plant growth conditions and varying fruit sizes make it hard to ensure that every animal receives a consistent and protective amount of vaccine.

**59.** (C) Idiotypes
According to the Jerne immune network theory, the immune system functions through a series of internal interactions between different antibody types. The term idiotype refers to the unique set of antigenic determinants located specifically on the variable region of an antibody. These idiotypes distinguish one antibody from another and are the targets for the production of anti-idiotypic antibodies in the network.

**60.** (A) Disulfide bridges
Synthetic peptide vaccines often struggle to maintain the native three-dimensional conformation required for recognition by the immune system. To address this, chemical modifications can be introduced to stabilize their spatial structure. For example, the creation of disulfide bridges can lock the peptide into a specific shape that mimics the original antigen, thereby improving the induction of high-affinity and neutralizing antibodies.

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**79.** (B) Escherichia coli
Escherichia coli remains the most commonly utilized host for recombinant protein expression in the biotechnology and vaccine industries. Its popularity stems from its rapid growth rate, well-characterized genetics, and the availability of diverse tools for genetic manipulation. These features allow for the high-yield production of many antigens, making it a cost-effective and efficient platform for developing various types of vaccines.

**80.** (A) P-2, Q-1, R-3, S-4
Different vaccine vectors have unique characteristics that determine their clinical use. Fowlpox virus is an avian vector that can express antigens in mammalian cells without fully replicating. Attenuated Salmonella is effective for oral mucosal targeting. Modified Vaccinia Ankara is a highly safe, non-replicating poxvirus, and recombinant adenoviruses are powerful tools often limited by pre-existing immunity in the host population.

**81.** (A) Absence of virulence sequences, making recombination improbable
Traditional live vaccines carry a small risk of reverting to a virulent state in the field. To eliminate this possibility, researchers use targeted gene deletion to create rationally attenuated strains. By completely removing the specific sequences responsible for virulence, it becomes virtually impossible for the pathogen to regain its original harmful properties through simple mutation or recombination, ensuring long-term safety.

**82.** (D) RNA molecules are intrinsically unstable and highly susceptible to ubiquitous RNases
While RNA vaccines offer several advantages, they also face significant technical challenges compared to DNA-based platforms. The most prominent limitation is that RNA molecules are intrinsically unstable and highly susceptible to degradation by ubiquitous RNases. This fragility necessitates the use of specialized delivery systems and strict cold-chain requirements during storage and transport to ensure the vaccine remains effective until administration.

**83.** (A) T-DNA (Transfer DNA)
The transformation of plant cells using Agrobacterium tumefaciens is a cornerstone of plant-based vaccine production. This process relies specifically on the transfer DNA region of the bacteria's Ti plasmid. The T-DNA is precisely excised and transferred into the plant cell, where it integrates into the nuclear genome. This allows the plant to permanently express the foreign antigen encoded within the sequence.

**84.** (C) I, III, and IV
Anti-idiotypic vaccines consist of antibodies that structurally mimic a pathogenic antigen and are particularly useful for targeting non-protein structures like carbohydrates. They provide a safe alternative to using virulent pathogens, although they have not yet seen widespread commercial success in veterinary medicine. These vaccines rely on the internal image of the variable region to stimulate a protective immune response in hosts.

**85.** (A) Linear peptides capable of binding to MHC molecules
In the design of synthetic peptide vaccines, it is essential to distinguish between different types of epitopes. T-cell epitopes are fundamentally different from B-cell epitopes because they must be processed and presented by MHC molecules. Therefore, T-cell epitopes are generally linear peptides rather than complex three-dimensional structures. This linear nature allows them to fit precisely into the binding cleft of MHC.

**86.** (C) Positive marker strategy
A marker vaccine strategy can utilize different approaches to distinguish between vaccinated and infected animals. A positive marker strategy involves the insertion of a foreign, highly immunogenic tag, such as green fluorescent protein, into the vaccine strain. The presence of antibodies against this unique tag confirms that the animal has been vaccinated, providing a clear signal that is distinct from infection.

**87.** (A) Neisseria meningitidis serogroup B
Reverse vaccinology achieved its first major clinical success with the development of an effective vaccine against Neisseria meningitidis serogroup B. Traditional methods had failed because the pathogen's capsule resembled human tissues. By analyzing the bacterial genome, researchers identified previously unknown surface-exposed proteins that could serve as protective antigens, demonstrating the power of this genome-based approach for difficult-to-target pathogens.

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responses, which are particularly effective at clearing intracellular infections and certain viral pathogens.

**18.** (D) They provide controlled release and are biodegradable
Polymeric PLGA microspheres are highly valued as particulate delivery systems because they are both biocompatible and biodegradable. These structures allow for the controlled and sustained release of the encapsulated antigen over an extended period. This prolonged exposure can reduce the need for multiple booster doses while protecting the antigen from premature degradation by host enzymes after administration into the body.

**19.** (A) VLPs lack genetic material and cannot replicate
Virus-like particles consist of viral structural proteins that spontaneously assemble into a configuration resembling the actual virus. However, they are fundamentally different from live viruses because they lack the genetic material required for replication. This makes them extremely safe while still presenting the immune system with a highly organized, repetitive display of native conformational epitopes that effectively induce protection.

**20.** (C) They scale easily in bioreactors without microcarriers
In large-scale industrial vaccine production, suspension cell cultures offer significant logistical advantages over adherent cultures. These cells grow freely in liquid media, making it much easier to scale up production using large stirred-tank bioreactors. This method eliminates the need for microcarriers or specialized surfaces for cell attachment, thereby simplifying the manufacturing process and increasing the overall yield of antigens.

**21.** (A) Prevention of ice formation and protection of structure
During the lyophilization or freeze-drying of a vaccine, the removal of water can damage the delicate structure of proteins or viruses. Cryoprotectants like sucrose or trehalose are added to the formulation to stabilize these biological components. These sugars replace water molecules through hydrogen bonding and prevent the formation of destructive ice crystals, ensuring the product remains potent upon reconstitution.

**22.** (D) Overdose administration and repeated administration
Target animal safety testing is a regulatory requirement that ensures a new vaccine is safe for its intended species. These evaluations must include the administration of an overdose, such as ten times the recommended dose, as well as repeated administrations. Monitoring for local and systemic adverse reactions under these extreme conditions provides necessary evidence of the product's safety profile.

**23.** (B) Field efficacy trials use gnotobiotic animal models
Pre-clinical development involves several critical stages, including immunogenicity trials to assess the immune response and challenge studies to measure protection. Dose determination is also essential for optimizing the antigen amount. However, field efficacy trials are not conducted using gnotobiotic or germ-free models; instead, they are performed under natural conditions with real-world exposure to pathogens to evaluate performance.

**24.** (C) Sterility and purity testing
Following production, every batch of a viral vaccine must undergo rigorous quality control to ensure its safety and effectiveness. Sterility and purity testing are mandatory procedures used to confirm the total absence of extraneous agents such as bacteria, fungi, mycoplasma, or adventitious viruses. These tests are vital for preventing the accidental introduction of pathogens into vaccinated animal populations during administration.

**25.** (C) A is true but R is false.
Toxoid vaccines consist of chemically inactivated exotoxins that have lost their toxicity but retain their ability to induce an immune response. Because these purified proteins are often poorly immunogenic on their own and do not naturally stimulate toll-like receptors, they require the addition of an adjuvant. This combination is necessary to create a robust and long-lasting defense against bacterial toxins.

**26.** (A) Natural passive immunity
Natural passive immunity is established when a newborn animal receives antibodies directly

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**97.** (B) The mandated time between vaccine administration and slaughter for human consumption
In veterinary medicine, the withdrawal period is a strictly regulated timeframe that must be followed after administering a vaccine or drug to a food-producing animal. It is defined as the mandated interval between the last administration and the time when the animal can be slaughtered for human consumption. This ensures that any residues or inflammatory responses have cleared from the tissues.

**98.** (C) The specific avian species for which the vaccine is intended (e.g., chickens)
When conducting clinical trials for a new veterinary vaccine, such as one for avian influenza, it is a regulatory requirement that safety must be evaluated in the target species. This means the vaccine must be tested in chickens or the specific avian species for which it is intended. Using the actual target animal ensures that species-specific biological responses and potential risks are accurately identified.

**99.** (B) Bacterial endotoxins (lipopolysaccharides)
The Limulus amebocyte lysate assay is a vital post-production quality control test used to ensure vaccine safety. This highly sensitive test is designed specifically to detect the presence of bacterial endotoxins, which are lipopolysaccharides found in the cell walls of Gram-negative bacteria. Ensuring the absence of these pyrogens is essential for preventing severe inflammatory reactions or fever in vaccinated animals.

**100.** (A) Both A and R are true and R is the correct explanation of A.
Recombinant protein subunit vaccines are generally considered safer than live-attenuated vaccines, especially for immunocompromised animals. This is because these vaccines consist only of purified antigenic components and totally lack the genetic material or capacity to replicate. Therefore, there is no risk of the vaccine causing disease or reverting to virulence, providing a high level of safety for vulnerable populations.

**101.** (A) Memory B cells and Memory T cells
The long-term protection provided by successful vaccination is the result of immunological memory. This memory primarily resides within two specific cellular compartments: memory B cells and memory T cells. These specialized cells are primed during the initial immunization and can persist for many years, allowing the immune system to respond with incredible speed and magnitude if the pathogen is encountered again.

**102.** (B) Treatment with heat or formalin to eliminate toxicity while maintaining immunogenicity
Converting a dangerous bacterial exotoxin into a safe toxoid for vaccination requires a specific chemical or physical treatment. The most common methodology involves treating the toxin with heat or formalin. This process effectively eliminates the toxin's harmful biological activity while maintaining its immunogenic epitopes. The resulting toxoid can then be safely administered to stimulate a protective immune response against the original toxin.

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**103.** (A) Perforin-mediated lysis of the bacteria
Humoral immunity provides several mechanisms to defend against extracellular bacterial pathogens, including the neutralization of toxins, opsonization for phagocytosis, and the activation of the complement cascade. However, perforin-mediated lysis is not a primary humoral mechanism; instead, it is a key function of cytotoxic T cells and natural killer cells used to destroy infected or abnormal host cells during a cellular immune response.

**104.** (C) To prevent the antigen from being expressed until the cell culture reaches optimal density
In eukaryotic expression systems used for vaccine production, inducible promoters are often preferred for controlling gene expression. These promoters allow researchers to delay the production of the antigen until the cell culture has reached its optimal density. This strategy is particularly useful if the antigen is toxic to the cells, as it prevents premature cell death and maximizes the overall yield.

**105.** (B) It eliminates the risk of integration into the host's nuclear genome
Poxviruses are unique among DNA viruses because they complete their entire replication cycle within the cytoplasm of the host cell. This characteristic provides a significant safety advantage for their use as vaccine vectors. Since the virus does not enter the cell nucleus, there is no risk of its genetic material integrating into the host's chromosomal DNA, thereby avoiding potential insertional mutagenesis.

vaccines are highly safe because they do not contain live pathogens and cannot cause disease. Furthermore, they are more thermostable than many traditional vaccines and are often administered using advanced methods like electroporation or a gene gun to ensure the DNA effectively enters the host's cells for expression.

**158.** (B) They mediate the excision, transfer, and integration of the T-DNA into the plant genome
In the context of plant-based vaccines, the virulence genes of the Agrobacterium Ti plasmid play a critical role. These genes are not part of the final vaccine antigen; instead, they encode the machinery necessary for the excision, transfer, and integration of the T-DNA into the plant genome. This process ensures that the target gene is stably incorporated into the plant's DNA for protein production.

**159.** (B) Ab3 (which neutralizes the actual pathogen)
The Jerne immune network theory explains how anti-idiotypic antibodies can be used to generate immunity. When an Ab2 antibody acts as an internal image of a pathogen's antigen, it can stimulate the production of Ab3 antibodies in the host. Because Ab2 mimics the antigen, these resulting Ab3 antibodies are functionally capable of recognizing and neutralizing the actual pathogen, providing a safe form of protection.

**160.** (A) Synthesize overlapping peptides derived from the antigen sequence, screen peptides with neutralizing sera, identify strongly binding peptide fragments, optimize peptide sequence and conformation
Mapping B-cell epitopes for a synthetic vaccine follows a specific sequence. It begins with the synthesis of overlapping peptides from the antigen. These are then screened with neutralizing sera to find the strongly binding fragments. Once identified, the peptide sequence and its conformation are optimized to ensure the final vaccine can effectively induce high-affinity antibodies that can neutralize the original pathogen in the host.

**161.** (B) Antibodies against $N_2$
In a DIVA strategy for avian influenza, the choice of antigens is critical for differentiation. If a vaccine contains $H_5$ and $N_1$, while the wild-type circulating virus is $H_5N_2$, the $N_2$ protein becomes the negative marker. Diagnostic tests will look for antibodies against $N_2$; their presence indicates natural infection, whereas vaccinated animals will only have antibodies against the $H_5$ and $N_1$ components of the vaccine.

**162.** (A) Surface-exposed or actively secreted
Reverse vaccinology is built on the fundamental principle that the most effective vaccine targets are those that the immune system can easily see. Therefore, researchers use genomic screening to identify proteins that are either surface-exposed on the bacterial cell or actively secreted into the surrounding environment. These proteins are the most likely candidates to be accessible to antibodies and to stimulate a protective response.

**163.** (A) Binding to a host cell receptor and/or changes in endosomal pH
The fusion glycoproteins of enveloped viruses are complex machines that must undergo a dramatic conformational change to allow the virus to enter a host cell. In a natural infection, this process is typically triggered by the binding of the virus to a specific host cell receptor or by the drop in pH that occurs after the virus is taken into an endosome.

**164.** (A) A specific immune response parameter that is statistically associated with protection against clinical disease
In the field of Vaccinomics, a correlate of protection is an essential metric used to evaluate vaccine success. It is defined as a specific immune response parameter, such as a particular antibody titer or T-cell count, that is statistically associated with protection against clinical disease. Identifying these correlates allows researchers to measure vaccine efficacy more accurately without always needing to conduct expensive challenge studies.

**165.** (C) A gene encoding an innate immune sensor, such as a Toll-Like Receptor (TLR)
Adversomics researchers investigate the genetic basis of adverse vaccine reactions by looking for specific markers in the host genome. A likely target for such studies would be single nucleotide polymorphisms in genes that encode innate immune sensors, such as Toll-Like Receptors. These variations can influence how the body initially responds to a vaccine, potentially leading to individual differences in safety and inflammatory reactions.

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different cell populations respond to a vaccine, helping to identify correlates of protection.

**115.** (D) IgE
Anaphylaxis is a severe and immediate adverse vaccine reaction that belongs to the Type I hypersensitivity category. This reaction is mediated by immunoglobulin E antibodies that have become cross-linked on the surface of mast cells and basophils. This triggering leads to the massive release of inflammatory mediators like histamine, which can cause systemic effects such as hypotension and respiratory distress in animals.

**116.** (A) They intercalate into cell membranes, creating pores that facilitate cytosolic delivery of antigens for CD8+ T cell activation
Saponin derivatives like QS-21 function as potent vaccine adjuvants through a specific cellular mechanism. They intercalate into the host cell membranes, creating temporary pores that facilitate the delivery of antigens directly into the cytosol. This pathway is essential for ensuring that the antigens are processed and presented on MHC Class I molecules, which is required for the activation of cytotoxic CD8+ T cells.

**117.** (B) Dendritic cells
Granulocyte-Macrophage Colony-Stimulating Factor is frequently used as a biological vaccine enhancer to improve the adaptive immune response. Its primary function is to target and promote the recruitment, maturation, and activation of dendritic cells. Since these are the most efficient antigen-presenting cells, their enhanced activity leads to more effective priming of T cells and a more robust overall response to the vaccine.

**118.** (A) To enhance electrostatic interaction with negatively charged DNA or RNA molecules
When designing liposomal delivery systems for vaccines, the inclusion of cationic lipids serves a specific purpose. These positively charged molecules enhance the electrostatic interaction with negatively charged nucleic acids, such as plasmid DNA or messenger RNA. This ensures efficient encapsulation and improves the ability of the liposome to bind to and enter host cells, thereby increasing the overall expression of the antigen.

**119.** (A) It tightly opens tight junctions between epithelial cells, increasing paracellular permeability
Chitosan is a widely used polymer in nanotechnology for targeted mucosal vaccine delivery. One of its key biological advantages is its ability to transiently open the tight junctions between epithelial cells. This increases the paracellular permeability of the mucosal barrier, allowing the vaccine antigens to pass through and reach the underlying lymphoid tissues more effectively, which results in a stronger immune response.

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**120.** (C) Inactivation agents like formaldehyde are purposefully left in high concentrations in the final product
Downstream processing involves several steps to purify the vaccine antigen, such as centrifugation to remove debris and ultrafiltration for concentration. Chromatography is also used to separate the target protein based on its properties. However, it is incorrect to suggest that inactivation agents like formaldehyde are left in high concentrations; they are carefully monitored and must be reduced to safe, minimal levels.

**121.** (A) Vacuum conditions and low temperature to allow sublimation of ice
In the process of lyophilization, primary drying is the stage where the majority of water is removed from the frozen product. This occurs under vacuum conditions and at a low temperature to facilitate the sublimation of ice directly into vapor. This careful removal of moisture is essential for preserving the delicate structural integrity of the vaccine's biological components while ensuring long-term stability.

**122.** (A) That the seed virus/bacteria used for production batches is limited to a strict number of passages to maintain genetic stability
The Master Seed concept is a fundamental regulatory requirement for ensuring the consistency and safety of vaccine production. It mandates that the specific virus or bacteria used for all production batches is limited to a strict number of passages from the original seed. This prevents the accumulation of genetic mutations over time, ensuring that every batch of vaccine has the same properties.

Differences in HLA or MHC molecules can explain why individuals respond differently to the same vaccine. Furthermore, polymorphisms in cytokine receptor genes are also important targets of study. This research aims to develop personalized safety guidelines to better protect genetically susceptible subpopulations.

**141.** (D) Squalene
Modern vaccine adjuvants like MF59 are designed to significantly boost the host's immune response, particularly for influenza vaccines. This specific adjuvant is a oil-in-water emulsion that contains squalene, a naturally occurring oil found in plants and animals. Squalene-based emulsions are effective at promoting the recruitment of immune cells to the injection site, leading to a more robust and persistent antibody response.

**142.** (A) Transforming Growth Factor-beta (TGF-beta)
To enhance the mucosal immunity of an intranasally delivered vaccine, researchers may include specific immunomodulators in the formulation. Transforming Growth Factor-beta is a rational choice for this purpose because it is a key cytokine that promotes IgA class switching in B cells. Increasing local IgA production at the mucosal surface provides a more effective defense against pathogens that enter through the respiratory tract.

**143.** (B) ISCOMs possess an open, cage-like structure formed by saponin, lipid, and antigen
Immune Stimulating Complexes are distinguished from simple liposomes by their unique and highly organized architecture. They possess an open, cage-like structure that is formed through the specific interaction of saponins, cholesterol, and phospholipids with the target antigen. This complex design not only stabilizes the antigen but also provides a potent stimulus that effectively activates both the humoral and cellular immune systems.

**144.** (B) The expression of one or more viral structural proteins capable of oligomerization
Virus-like particles are a powerful vaccine platform because they mimic the structure of a real virus without being infectious. The fundamental requirement for their production is the expression of one or more viral structural proteins that are capable of spontaneous selF-assembly. Once expressed in a suitable system, these proteins oligomerize into a geometric configuration that presents the immune system with native conformational epitopes.

**145.** (A) Cell lysis/harvesting, clarification (filtration/centrifugation), purification (chromatography), concentration and formulation
The downstream processing of viral vaccines produced in cell culture involves a specific sequence of steps to ensure a high-quality product. It begins with cell lysis and harvesting, followed by clarification through filtration or centrifugation to remove debris. Next, the antigen is purified using techniques like chromatography. Finally, the product is concentrated and formulated with the necessary stabilizers and adjuvants.

**146.** (C) Secondary drying (desorption)
Lyophilization is a complex process that ensures the long-term stability of many vaccines. The final stage is known as secondary drying or desorption. During this step, the temperature is slightly increased under a high vacuum to remove the small amount of residual moisture that remains tightly bound to the biological material. This is critical for preventing chemical degradation and extending the product's shelf life.

**147.** (B) A legal document certifying that a specific batch meets all established quality, purity, and potency specifications
A Certificate of Analysis is a vital legal document used in the regulated manufacture of vaccines. It provides official certification that a specific batch of the product has undergone rigorous testing and meets all established specifications for quality, purity, and potency. This document ensures that every dose administered to animals is safe and will perform as expected according to regulatory standards.

**148.** (C) Pharmacokinetic studies of the antigen are usually as extensive as those required for chemical drugs
Clinical trials for veterinary vaccines follow structured phases to evaluate performance. While laboratory efficacy and field trials are mandatory, it is incorrect to assume that pharmacokinetic studies of vaccine antigens are as extensive as those for chemical drugs. Because vaccines act by stimulating the immune system rather than through direct chemical action, the

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to remove selectable markers. These tools facilitate the rational design of vaccines.

**132.** (D) It evades recognition by innate immune sensors (like TLR7) and increases translation efficiency
The inclusion of modified nucleosides, such as pseudouridine, is a critical innovation in RNA vaccine technology. These modifications allow the mRNA to evade detection by innate immune sensors like Toll-like receptor 7. By reducing the immediate inflammatory response to the foreign RNA, these modified nucleosides significantly increase the efficiency of translation, leading to higher levels of antigen production and immunity.

**133.** (B) Plant-based molecular farming
Plant-based molecular farming is an innovative approach to large-scale antigen production that does not rely on traditional tissue culture or fermentation equipment. By using whole plants as bioreactors, this technology offers a cost-effective and scalable alternative to mammalian or bacterial systems. This method also provides added biosafety, as plants do not harbor mammalian viruses that could contaminate the final vaccine.

**134.** (D) It possesses highly toxic or difficult-to-purify carbohydrate antigens
Anti-idiotypic vaccines provide a unique solution for pathogens that possess antigens that are difficult to work with, such as toxic or hard-to-purify carbohydrates. By using an antibody that mimics the structure of these antigens, researchers can stimulate a protective immune response without ever needing to handle the actual pathogenic molecules. This makes the production process safer and more manageable for certain diseases.

**135.** (A) Linear (continuous) and conformational (discontinuous)
B-cell epitopes, which are the specific parts of an antigen recognized by antibodies, are classified into two main categories based on their structure. Linear or continuous epitopes consist of a simple sequence of amino acids. In contrast, conformational or discontinuous epitopes are formed by amino acids that are brought together by the complex three-dimensional folding of the protein, making their mimicry more challenging.

**136.** (C) Envelope glycoprotein $E_2$
The eradication of Classical Swine Fever relies heavily on a DIVA strategy that incorporates a specific recombinant subunit vaccine. This vaccine is based on the envelope glycoprotein $E_2$, which is highly immunogenic and provides strong protection. Because the vaccine only contains the $E_2$ protein, diagnostic tests can look for antibodies against other viral proteins to identify naturally infected pigs in a population.

**137.** (A) The study of the entire core and accessory genomes of all strains within a species
In the field of reverse vaccinology, pan-genomics refers to the study of the entire genetic repertoire of a bacterial species. This includes both the core genome, which consists of genes shared by all strains, and the accessory genome, which contains genes found in only some strains. Analyzing the pan-genome allows researchers to identify conserved antigens that can provide broad protection.

**138.** (D) Introducing engineered disulfide bonds and proline substitutions to lock the pre-fusion conformation
A major focus of structural vaccinology is the optimization of viral glycoprotein antigens to maximize their immunogenicity. This is achieved by introducing engineered disulfide bonds and proline substitutions to lock the proteins into a stable pre-fusion conformation. This approach prevents the proteins from undergoing conformational changes that would otherwise hide the most important neutralizing epitopes from the host's immune system.

**139.** (A) Artificial neural networks for MHC binding affinity prediction (e.g., NetMHCpan)
Systems vaccinology utilizes advanced algorithms to analyze large-scale genomic data and predict the most effective vaccine targets. Artificial neural networks, such as NetMHCpan, are frequently used to predict the binding affinity of different peptides to MHC Class I and II molecules. Identifying these high-affinity epitopes is a critical step in designing vaccines that can effectively activate T-cell responses in diverse populations.

**140.** (B) I, III, and IV
Adversomics is a critical field that examines how host genetic variation influences the risk of adverse reactions following vaccination.

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them with a known virulent strain of the pathogen. By measuring the level of protection provided, manufacturers can confirm that the batch will be effective in the field.

**175.** (A) Both A and R are true and R is the correct explanation of A.
DNA vaccines can be made significantly more immunogenic by including unmethylated CpG motifs in their design. These motifs act as natural adjuvants because they are recognized by Toll-Like Receptor 9 in the immune system. This interaction triggers a potent activation of the innate immune response and helps steer the adaptive response toward a Th1 profile, which is vital for effective cell-mediated immunity.

**176.** (A) Live-attenuated vaccines
Of all the traditional vaccine platforms, live-attenuated vaccines induce an immune response that most closely resembles the one generated by a natural infection. Because the vaccine contains a living organism that replicates within the host, it provides a prolonged and diverse antigenic stimulus. This effectively activates both humoral and cell-mediated pathways, often resulting in strong, long-lasting immunity with fewer doses than killed vaccines.

**177.** (A) They may cause an outbreak of the actual disease
The primary risk associated with the manufacture of traditional killed viral vaccines is the potential for incomplete inactivation. If the chemical or physical treatment fails to destroy all infectious particles, the vaccine could inadvertently cause an outbreak of the actual disease when administered. This highlights the absolute necessity for rigorous quality control and validation of the inactivation process to ensure every batch is safe.

**178.** (B) Cytokine signals from T-helper cells orchestrating class switch recombination
During an immune response, B cells can change the type of antibody they produce through a process called class switch recombination. This switch from the initial IgM to other isotypes like IgG or IgA is strictly determined by cytokine signals received from T-helper cells. These signals orchestrate the genetic changes needed for the B cell to produce antibodies that are better suited for specific environments.

**179.** (A) I, II, and IV
Poxvirus vectors are versatile tools for vaccine delivery because they have a large capacity for foreign DNA and replicate entirely in the host cell's cytoplasm. This cytoplasmic replication is a key safety feature as it prevents the risk of the vector integrating into the host's nucleus. Furthermore, specialized vectors like canarypox are particularly safe because they undergo an abortive infection in mammalian species.

**180.** (A) Aromatic amino acids (e.g., tryptophan, tyrosine, phenylalanine)
Rationally attenuated bacterial vaccines can be created by knocking out the aroA gene, which is essential for synthesizing aromatic amino acids like tryptophan and phenylalanine. Because mammals do not have the metabolic pathways to produce these specific nutrients, the bacteria cannot find them within the host's tissues. This metabolic restriction ensures that the vaccine strain cannot replicate excessively, making it safe for use.

**181.** (A) It stabilizes the transcribed mRNA and facilitates its export from the nucleus for translation
In the construction of DNA vaccines, the inclusion of a polyadenylation signal sequence is vital for successful antigen production. This regulatory element ensures that the transcribed mRNA is stable and can be efficiently exported from the host cell nucleus into the cytoplasm. Once in the cytoplasm, the mRNA can then be translated by ribosomes into the protein antigen that will trigger the immune response.

**182.** (A) They harbor zero risk of contamination with mammalian viral pathogens or prions
One of the most significant biosafety advantages of using plant expression systems for vaccine production is the elimination of certain risks associated with animal-based systems. Plants do not harbor mammalian viral pathogens or prions, so there is no possibility of these dangerous agents contaminating the final vaccine product. This inherent safety simplifies the manufacturing process and reduces the risk of accidental disease transmission during immunization.

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regulatory requirements for their distribution and metabolism are different.

**149.** (A) Hemagglutinin (HA)
The single radial immunodiffusion assay is a classic in vitro potency test used in the quality control of influenza vaccines. This technique is specifically designed to quantify the mass of the hemagglutinin antigen, which is the primary component responsible for inducing protective immunity. Ensuring the correct amount of HA in each batch is essential for the vaccine to be effective in the field.

**150.** (A) Both A and R are true and R is the correct explanation of A.
Plant-based edible vaccines offer a significant logistical advantage because they may circumvent the need for an expensive cold chain. The antigens expressed in tissues like seeds or tubers are often naturally protected from thermal degradation by the rigid plant cell wall and internal compartmentalization. This inherent stability makes these vaccines particularly suitable for use in regions where refrigerated transport and storage are unavailable.

**151.** (A) The harsh acidic environment of the stomach and proteolytic enzymes in the gut
The success of orally administered live-attenuated vaccines is often hindered by the harsh conditions of the gastrointestinal tract. The primary barrier is the highly acidic environment of the stomach combined with various proteolytic enzymes in the gut, which can rapidly degrade or inactivate the vaccine organisms. Overcoming these challenges is essential for ensuring that enough of the vaccine survives to reach the lymphoid tissues.

**152.** (B) Pathogens whose primary pathogenesis is mediated by secreted exotoxins (e.g., Clostridium tetani)
Toxoid vaccines are a highly effective category of immunization specifically designed to target bacterial pathogens whose primary mechanism of disease is the secretion of exotoxins. Classic examples include Clostridium tetani and Clostridium botulinum. By stimulating the production of neutralizing antibodies against these toxins, the vaccine prevents the severe clinical signs associated with the infection, even if the bacteria themselves are present.

**153.** (B) Endogenous (cytosolic) pathway and presented on MHC Class I
To induce a robust CD8+ T cell response, a vaccine antigen must be processed and presented through a specific cellular pathway. This occurs when the antigen is present in the host cell's cytosol, allowing it to enter the endogenous pathway. Here, the protein is degraded by proteasomes and then presented on MHC Class I molecules, which is the signal required for the activation of cytotoxic T cells.

**154.** (B) Hyper-mannosylation of glycoproteins, which may alter antigenicity
Saccharomyces cerevisiae is a common eukaryotic system for vaccine production, but it has a significant limitation regarding the quality of the antigens produced. This yeast often performs hyper-mannosylation, which involves adding excessive mannose chains to glycoproteins. This unnatural glycosylation pattern can alter the antigenicity of the protein and potentially interfere with the induction of a protective immune response in the host animal.

**155.** (A) P-2, Q-4, R-1, S-3
Modern veterinary medicine utilizes several recombinant platforms. A classic example of a poxvirus vector is the Raboral V-RG vaccine for rabies. The gE-deleted BoHV-1 is a well-known DIVA mutant, and the $E_2$ glycoprotein marker vaccine is used for Classical Swine Fever. Additionally, DNA vaccines have been developed for West Nile Virus in horses. These examples illustrate the diverse applications of genetic engineering.

**156.** (A) The bacteria exhibit impaired survival and replication in host tissues due to metabolic deficiency
Creating a rationally attenuated bacterial vaccine involves specific genetic modifications to ensure safety. For example, knocking out the adenylate cyclase gene in Salmonella impairs the pathogen's ability to survive and replicate effectively within host tissues. This metabolic deficiency ensures that the bacteria are weakened enough to prevent disease while still being able to stimulate a strong and protective immune response in the animal.

**157.** (B) I, III, and IV
DNA vaccines consist of a bacterial plasmid that carries the gene for a specific antigen. These

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**123.** (B) Efficacy and safety under natural field conditions involving a large number of target animals
Phase III clinical trials represent the final and most extensive stage of evaluation for a new veterinary vaccine before it can be licensed. These trials are designed to establish the product's efficacy and safety under natural field conditions, involving a large number of target animals across diverse geographical locations. This large-scale testing provides the necessary statistical evidence of the vaccine's performance in real-world scenarios.

**124.** (A) Hemagglutination inhibition or Virus Neutralization Test
Assessing the potency of a vaccine batch is critical for ensuring that it will provide protection in the field. To specifically measure the levels of neutralizing antibodies induced by the vaccine, researchers utilize functional assays such as the Hemagglutination Inhibition test or the Virus Neutralization Test. These assays provide a direct measurement of the immune system's ability to block the pathogen.

**125.** (A) Both A and R are true and R is the correct explanation of A.
Edible plant-based vaccines face a unique immunological challenge known as oral tolerance. The gut-associated lymphoid tissue has evolved to suppress systemic immune responses to harmless dietary proteins to prevent food allergies. Therefore, a vaccine administered this way must be carefully designed to overcome this natural suppression and instead stimulate a protective response, which is a major hurdle for this technology.

**126.** (A) Immunological memory
Active immunization involves stimulating the host's own immune system with an antigen, which offers a distinct long-term advantage over passive immunization. The primary benefit is the generation of immunological memory, consisting of specialized B and T cells. Unlike passive immunization, which only provides temporary protection through transferred antibodies, active immunity allows the animal to mount its own defense upon future exposure.

**127.** (B) Formalin (formaldehyde)
Chemical inactivation is a critical step in producing killed vaccines, and formalin is the agent most commonly used for this purpose. It works by cross-linking proteins, which effectively destroys the pathogen's ability to replicate or cause disease. A major benefit of using formalin is that it preserves the structural integrity of surface epitopes, ensuring that the immune system can still recognize them.

**128.** (A) They secrete IFN-gamma and IL-2 to activate macrophages and cytotoxic T cells
During a viral infection, the T-helper 1 response is vital for orchestrating an effective defense. These cells secrete critical cytokines like Interferon-gamma and Interleukin-2, which activate macrophages and promote the development of cytotoxic T cells. This cell-mediated pathway is essential for identifying and destroying host cells that have been hijacked by the virus, thereby limiting the spread of the infection.

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**129.** (A) I, II, and IV
Recombinant subunit vaccines are highly safe because they consist of isolated proteins or glycoproteins rather than whole infectious agents, meaning they cannot revert to a virulent state. However, their efficacy is extremely dependent on the correct folding of the antigen to mimic the native pathogen. Because they are often less immunogenic than live vaccines, they typically require the use of adjuvants.

**130.** (B) They may cause disease in non-target or immunocompromised animals
A primary safety concern that limits the widespread use of replication-competent viral vectors in veterinary medicine is their potential impact on vulnerable populations. While effective, these vectors could potentially cause disease in non-target species or immunocompromised animals if they are shed into the environment. This risk necessitates careful assessment and often leads to a preference for replication-defective or highly attenuated vector systems.

**131.** (A) P-2, Q-1, R-3, S-4
Modern vaccinology utilizes several genetic manipulation tools to engineer pathogens. CRISPR/Cas9 allows for precise targeted gene editing, while reverse genetics enables the rescue of infectious RNA viruses from cloned cDNA. Plasmid transfection is used to introduce DNA into eukaryotic cells, and the Cre/loxP system provides a method for site-specific recombination

**166.** (C) Cytotoxic CD8+ T cell (Th1) responses
Aluminum-based adjuvants are the most common additives in veterinary vaccines, but they have a notable immunological limitation. While they are excellent at promoting Th2-biased responses and high antibody titers, they are generally poor at inducing potent cytotoxic CD8+ T cell or Th1-type immunity. This makes them less suitable for vaccines that require a strong cell-mediated defense to clear intracellular pathogens or certain viral infections.

**167.** (C) No potential risk for systemic toxicity or cytokine storm
Using cytokines as biological vaccine enhancers offers several benefits, such as skewing the response toward a Th1 or Th2 profile. However, it is incorrect to suggest that there is no potential risk associated with their use. Inappropriate delivery or excessive levels of these potent signaling molecules can lead to systemic toxicity or a cytokine storm, which could be harmful or even fatal to the animal.

**168.** (A) Hydrolysis of their ester linkages
Poly-lactic-co-glycolic acid is a widely used material for creating biodegradable vaccine microspheres. Once administered into the host's body, these structures begin to break down through a predictable chemical process. The primary mechanism of degradation is the hydrolysis of the ester linkages in the polymer backbone. This gradual breakdown allows for the sustained release of the encapsulated antigen as the microspheres slowly disappear.

**169.** (B) The repetitive, high-density display of conformational epitopes on a geometric surface
Virus-like particles are exceptionally immunogenic, even without the presence of a viral genome. Their high potency is due to their unique structural organization, which features a repetitive, high-density display of native conformational epitopes on a geometric surface. This arrangement effectively cross-links B-cell receptors and provides a strong signal to the immune system, leading to a much more robust response than soluble monomeric proteins.

**170.** (A) Lactate and glucose concentrations in the media
In the large-scale production of viral vaccines, monitoring the metabolic state of the cell culture is essential for success. Operators routinely measure the concentrations of key molecules like lactate and glucose within the media. These measurements provide a clear picture of cell growth and health, allowing for precise adjustments to be made to the feeding schedule or bioreactor conditions to maximize the final yield.

**171.** (A) To provide an elegant, structurally sound cake appearance and physical support
Bulking agents like mannitol are critical components of lyophilized vaccine formulations. Their primary role is to provide physical support and ensure the creation of an elegant, structurally sound cake appearance after the water is removed. Without these agents, the dried product might collapse or become a dense, difficult-to-dissolve mass, which would negatively impact the stability and ease of use of the vaccine.

**172.** (B) That a live-attenuated vaccine strain does not become pathogenic after repeated serial passages in the target host
The reversion to virulence test is a mandatory safety evaluation for all live-attenuated vaccines under pharmacopeial guidelines. This test is designed to demonstrate that the vaccine strain remains stable and does not regain its pathogenic properties after being passed repeatedly through the target host species. This ensures that the vaccine will not cause an accidental outbreak of the disease after it is used.

**173.** (A) P-3, Q-4, R-1, S-2
Clinical trials for vaccines proceed through several stages with distinct objectives. Phase I focuses on initial safety in a small group, while Phase II involves dose determination and preliminary efficacy. Phase III consists of large-scale field trials to establish effectiveness under real-world conditions. Finally, Phase IV involves post-marketing surveillance to monitor for long-term adverse events after the vaccine has been licensed and distributed.

**174.** (D) Vaccinating a group of animals and subsequently challenging them with a virulent strain to measure protection
The in vivo potency test is a critical quality control procedure performed on individual batches of vaccines before they are released for sale. This procedure typically involves vaccinating a group of animals and then subsequently challenging

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**106.** (A) P-2, Q-4, R-1, S-3
Modern vaccinology uses various genetic tools to create safer and more effective products. Gene deletion mutants have specific virulence genes removed, while chimeric vaccines combine structural genes from different pathogens. Codon deoptimization utilizes non-preferred sequences to reduce viral translation, and DNA vaccines use plasmids driven by mammalian promoters. These techniques allow for the rational design of a wide range of vaccines.

**107.** (B) Lipid nanoparticles (LNPs)
RNA vaccines require a specialized delivery vehicle to be effective, as naked mRNA is rapidly degraded and poorly taken up by cells. Lipid nanoparticles are the primary technology used for this purpose. These vehicles encapsulate the mRNA, protecting it from enzymes and facilitating its entry into the cytosol. Once inside, the host's own machinery can then translate the mRNA into the desired antigen.

**108.** (B) Chloroplast DNA is strictly maternally inherited in most plant species, excluding it from pollen
Chloroplast engineering is an attractive platform for plant-based vaccines due to its high expression levels and inherent biosafety features. In most plant species, chloroplast DNA is strictly maternally inherited. This means that the transgene is not present in the pollen, effectively preventing the accidental spread of the vaccine gene to wild relatives or neighboring crops, which is a major environmental concern.

**109.** (B) They are highly restricted by individual MHC haplotype variations and may induce tolerance
A major theoretical limitation of anti-idiotypic vaccines in large-scale applications is their relationship with the host's genetic background. These vaccines can be highly restricted by individual MHC haplotype variations, which means they may not be effective across an entire population. Furthermore, there is a risk that the immune system might develop tolerance toward these antibodies rather than mounting a protective response.

**110.** (A) I, II, and IV
Coupling synthetic peptides to large carrier proteins like Keyhole Limpet Hemocyanin is a common strategy to enhance immunogenicity. This process utilizes chemical cross-linkers such as glutaraldehyde to create a stable bond. The primary purpose of the carrier protein is to provide the necessary T-cell epitopes that are often missing from the peptide itself, thereby enabling a full and effective immune response.

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**111.** (D) Positive for gE antibodies
The gE-deleted Bovine Herpesvirus-1 vaccine is a classic example of a DIVA strategy. In this system, the vaccine strain lacks the gE protein. Therefore, an animal that has only been vaccinated will not have antibodies against gE. In contrast, an animal naturally infected with the wild-type virus will test positive for gE antibodies, allowing for easy differentiation between the two.

**112.** (D) The genomes of multiple strains of a pathogen to identify conserved, universally protective surface antigens
In the context of reverse vaccinology, pan-genomic screening involves the comprehensive analysis of the entire genetic repertoire of a pathogen species. By examining the genomes of multiple different strains, researchers can identify conserved antigens that are present in every version of the pathogen. These universally shared proteins are the ideal targets for developing vaccines that provide broad protection against all circulating strains.

**113.** (A) Pre-fusion state
Structural vaccinology research has shown that the configuration of viral antigens is crucial for their effectiveness. For the Respiratory Syncytial Virus, the F protein must be stabilized in its pre-fusion state to elicit the highest titer of neutralizing antibodies. This specific conformation exposes highly sensitive epitopes that are lost once the protein transitions to its post-fusion state during the infection process.

**114.** (D) Deep profiling of thousands of individual immune cells simultaneously based on multiple surface markers
In the field of Vaccinomics, high-throughput technologies like mass cytometry, or CyTOF, are used for deep immunological analysis. This powerful tool allows researchers to profile thousands of individual immune cells simultaneously based on a large number of surface and intracellular markers. This level of detail provides an unprecedented understanding of how

**183.** (B) Anti-idiotypic vaccines are particularly well-suited for easily cultivated, highly safe protein antigens
Anti-idiotypic vaccines are based on the Jerne immune network theory and utilize Ab2 antibodies to mimic the structure of an original antigen. While they are a fascinating approach, it is incorrect to suggest they are well-suited for easily cultivated protein antigens. Instead, their true value lies in targeting antigens that are difficult to produce or dangerous to handle, providing a safe alternative for complex pathogens.

**184.** (A) To prevent the epitopes from interfering with each other's independent folding and recognition
When designing synthetic peptide vaccines that contain multiple distinct epitopes, researchers often include spacer or linker sequences between them. The primary role of these spacers is to prevent the different epitopes from interfering with each other's independent folding and immune recognition. This structural separation ensures that each part of the peptide can be effectively processed and presented to the immune system as intended.

**185.** (A) Identify a non-essential highly immunogenic viral protein, delete the gene encoding this protein from the vaccine strain, develop an ELISA targeting the deleted protein, validate the ELISA to distinguish infected from vaccinated animals
The development of a successful DIVA strategy follows a logical series of molecular and diagnostic steps. First, a non-essential but highly immunogenic protein must be identified. Next, the gene for this protein is deleted from the vaccine strain. Then, a companion ELISA is developed to target the missing protein. Finally, the entire system is validated to ensure it can accurately distinguish between animals.

**186.** (B) High sequence conservation across multiple virulent strains
During the in silico screening phase of reverse vaccinology, researchers look for specific characteristics to identify the best vaccine candidates. An ideal antigen should have a high degree of sequence conservation across multiple virulent strains of the pathogen. This ensures that the resulting vaccine will provide broad protection against diverse versions of the disease rather than being effective against only a single strain.

**187.** (B) High-yield purification and generation of a stable, ordered protein crystal
Structural vaccinology utilizes X-ray crystallography to determine the precise three-dimensional structure of antigens at the atomic level. To perform this technique, researchers must first achieve high-yield purification of the target protein. They then must succeed in growing a stable and highly ordered protein crystal. This crystal is necessary to scatter the X-rays in a predictable pattern, allowing the structure to be solved computationally.

**188.** (B) Forecasting the robustness and longevity of the immune response based on early molecular signatures
Systems vaccinology uses sophisticated mathematical modeling to integrate complex data from multiple sources. The ultimate goal of this approach is to predict the robustness and longevity of a host's immune response shortly after vaccination. By identifying early molecular signatures that correlate with protection, researchers can forecast the long-term success of the vaccine and potentially identify which individuals may need additional booster doses.

**189.** (C) Type III
Type III hypersensitivity reactions are an important consideration in vaccine safety, particularly following repeated hyper-immunization. These reactions are caused by the formation and deposition of immune complexes in tissues, such as the skin or blood vessels. A classic example is the Arthus reaction, which can occur at the site of a vaccine injection. Understanding these mechanisms is a key part of adversomics research.

**190.** (A) P-2, Q-4, R-1, S-3
Advanced particulate systems offer diverse options for vaccine delivery. Liposomes are flexible vesicles for encapsulating various antigens, while PLGA nanoparticles provide controlled release. Virus-like particles are spontaneously assembled structures that mimic pathogens without a genome. Nanogels are unique hydrophilic networks that can respond to physiological triggers. Each platform has distinct characteristics that make it suitable for specific types of antigens and routes of administration.

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**191.** (B) TLR3
Poly(I:C) is a synthetic analog of double-stranded RNA that is frequently used as a potent vaccine adjuvant. It functions by specifically activating Toll-Like Receptor 3, which is a key component of the innate immune system. This activation mimics a viral infection, leading to the production of interferons and other cytokines that help stimulate a strong and effective adaptive immune response against the vaccine's antigen.

**192.** (A) CCL2 (MCP-1)
Chemokines are useful tools for enhancing the effectiveness of vaccines by directing the movement of immune cells. To attract critical antigen-presenting cells like dendritic cells and macrophages to the injection site, a formulator might include the chemokine CCL2, also known as MCP-1. This targeted recruitment ensures that the vaccine antigen is captured and processed more efficiently, leading to a much more powerful immune response.

**193.** (A) Local mucosal immunity mediated by secretory IgA and tissue-resident memory T cells
The primary rationale for using targeted mucosal delivery for vaccines against respiratory pathogens is to establish a strong local defense at the site of entry. This approach aims to induce high levels of secretory IgA and promote the development of tissue-resident memory T cells. By creating an immune barrier exactly where the pathogen first contacts the host, these vaccines can provide more effective protection.

**194.** (B) The harvesting, purification, concentration, and formulation of the product
In the context of industrial vaccine manufacturing, downstream processing refers to all the activities that occur after the antigen has been produced. This strictly includes harvesting the product from the bioreactor, followed by extensive purification and concentration steps. Finally, the purified antigen is formulated with adjuvants and stabilizers. This entire process is vital for ensuring the safety, purity, and potency of the final vaccine.

**195.** (A) I, II, and IV
Stabilizers are essential components of vaccine formulations that protect the antigen from degradation during storage. While historic stabilizers like gelatin carried certain risks, modern alternatives such as sucrose and trehalose are widely used and highly safe. These molecules are not removed before injection; instead, they remain in the final product to ensure that the vaccine remains potent from the time of manufacture until administration.

**196.** (A) The absolute absence of any living bacteria or fungi
Under pharmacopeial guidelines, the sterility test is a mandatory quality control procedure for every final batch of vaccine. This test is specifically designed to confirm the absolute absence of any living bacteria or fungi. Ensuring that the product is sterile is a fundamental safety requirement to prevent the accidental introduction of infections into animals when the vaccine is administered by a veterinarian or farmer.

**197.** (B) It evaluates if the induced immune response protects the animal against deliberate infection with a virulent pathogen
Establishing a challenge model is a critical part of pre-clinical efficacy trials for any new vaccine. This involves vaccinating a group of animals and then deliberately exposing them to a virulent strain of the pathogen. This process allows researchers to evaluate whether the immune response induced by the vaccine is truly protective against infection, providing the essential proof of concept needed before proceeding further.

**198.** (A) Both A and R are true and R is the correct explanation of A.
Real-time PCR is a powerful tool used in the post-production quality control of live-attenuated vaccines. This technique allows for the rapid and accurate quantification of the viral genome copy number in each batch. By ensuring that the correct amount of genetic material is present, manufacturers can verify that each vial contains the intended dose and meets the required standards for identity and consistency.

**199.** (B) Secondary response antibodies have progressively higher affinity due to affinity maturation
A key difference between the primary and secondary immune responses is the quality of the antibodies produced. During the secondary response, antibodies typically have a progressively higher affinity for the antigen than those produced initially. This improvement is the

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result of affinity maturation, a process driven by somatic hypermutation in B cells. This ensures that the immune system's defense becomes more precise and effective with repeated exposure.

**200.** (A) Natural passive immunity (maternal IgG)
In animals with hemochorial placentation, such as humans and some rodents, maternal IgG can be transferred directly to the fetus through the placenta, establishing natural passive immunity. However, in species with epitheliochorial placentation, like pigs and horses, the placental layers are too thick for antibody transfer. In these animals, the transfer of maternal antibodies can only occur after birth through the ingestion of colostrum.

**201.** (A) Formaldehyde
Formaldehyde has a long history of use in the creation of toxoid vaccines for diseases like tetanus. It is favored for this purpose because of its ability to cross-link proteins, which effectively destroys the biological toxicity of the original exotoxin. Crucially, this treatment allows the molecule to retain its immunogenic properties, enabling it to stimulate the production of protective neutralizing antibodies when given as a vaccine.

**202.** (A) P-2, Q-3, R-1, S-4
T-cell subsets play diverse and specialized roles in the immune response. Th1 cells support cell-mediated immunity, while Th2 cells drive defenses against helminths. T follicular helper cells are essential for B-cell activation and affinity maturation in germinal centers. Finally, regulatory T cells function to suppress excessive responses and maintain tolerance. Matching these subsets to their functions is key to understanding how the immune system is organized.

**203.** (B) E. coli lacks the cellular machinery for eukaryotic N- and O-linked glycosylation
Using Escherichia coli to express viral envelope proteins is challenging because the bacteria lack the complex machinery for eukaryotic post-translational modifications. This is particularly problematic for heavily glycosylated proteins, as E. coli cannot perform N- or O-linked glycosylation. Without these modifications, the resulting protein may not fold correctly or mimic the native structure of the virus, potentially reducing its effectiveness as a vaccine antigen.

**204.** (A) Oral administration
Recombinant Salmonella vectors are a versatile platform for vaccine delivery due to their natural tropism for the gut. To take full advantage of this characteristic, these vectors are typically administered via the oral route. This allows the bacteria to target the gut-associated lymphoid tissue, where they can effectively stimulate both local mucosal and systemic immune responses, making them particularly useful for vaccines against various enteric pathogens.

**205.** (D) They spontaneously regenerate the deleted gene when introduced into the host
Targeted gene-deletion mutants represent a sophisticated approach to creating rationally attenuated vaccines through precise molecular techniques. These mutants are highly stable and can serve as excellent DIVA vaccines. However, it is incorrect to suggest that they can spontaneously regenerate the deleted gene once they are in the host. Because the DNA sequence has been permanently removed, the risk of reversion to the wild-type state is virtually eliminated.

**206.** (A) The negative charge of the plasmid repelling the negatively charged cell membrane, and physical degradation by nucleases
The development of effective DNA vaccines is hindered by several significant biological barriers. Following injection, the plasmid DNA faces a negative charge on the host cell membrane that repels it, and it is also subject to rapid degradation by various nucleases in the extracellular environment. These factors severely restrict the amount of DNA that successfully enters the host cells, necessitating the use of specialized delivery methods.

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**207.** (B) The plant cell wall protecting the expressed antigen from rapid degradation in the stomach
The concept of bioencapsulation is a major benefit of using plant-based edible vaccines. It describes how the rigid cellulose cell wall of the plant naturally protects the expressed antigen from the harsh conditions of the animal's stomach. This protection prevents rapid proteolytic degradation, allowing the antigen to reach the intestinal lymphoid tissues intact. This natural delivery system simplifies the administration of vaccines and can improve their overall effectiveness.

**239.** (A) Th2 and robust humoral (antibody) production
Incorporating the cytokine Interleukin-4 into a vaccine formulation is a strategic way to steer the host's immune response in a specific direction. IL-4 is a potent driver of Th2 differentiation, which leads to a robust humoral response characterized by the production of high levels of antibodies. This profile is particularly useful for vaccines targeting extracellular pathogens or toxins where a strong antibody defense is the primary mechanism required for protection and clearance.

**240.** (A) P-4, Q-2, R-1, S-3
Different nanotechnology platforms have unique structural definitions for vaccine delivery. Liposomes are simple phospholipid bilayers, while virosomes incorporate active viral proteins. Virus-like particles are empty structures that mimic a pathogen's organization without any genome. Polymeric nanoparticles are solid matrix particles made from materials like PLGA. Each of these platforms offers different benefits for protecting antigens, improving uptake by immune cells, and controlling the release of the vaccine after administration into the body.

**241.** (A) Shifts in pH and the presence of specific degrading enzymes
Nanogels used for targeted mucosal vaccine delivery are designed to be responsive to their surroundings. A common stimulus that triggers the release of the vaccine antigen in the gut is a shift in pH or the presence of specific degrading enzymes. This environmental sensitivity ensures that the antigen is released exactly where it is needed most, leading to more efficient sampling by the immune system and the induction of a strong local defense.

**242.** (A) Constant removal of toxic metabolic byproducts and continuous supply of nutrients, leading to vastly higher cell densities
In large-scale vaccine manufacturing, switching from batch mode to continuous perfusion mode offers significant advantages for productivity. This method allows for the constant removal of toxic metabolic byproducts while continuously supplying fresh nutrients to the cells. This balanced environment leads to much higher cell densities and a more consistent production of the target antigen over an extended period, making the overall process more efficient and cost-effective for meeting high global demands.

**243.** (C) Freezing rates do not affect the viability of the live virus or the protein structure
Lyophilization is an essential process for stabilizing many vaccines, but it is incorrect to assume that freezing rates have no impact. In fact, both the speed of freezing and the subsequent desiccation stress can significantly affect the viability of live viruses and the delicate three-dimensional structure of proteins. Therefore, using specific lyoprotectants and carefully controlling the process parameters is critical for ensuring the final dry product remains potent and effective upon reconstitution for use.

**244.** (A) To provide a fully characterized, standardized, and pathogen-free cell line for all future production
According to regulatory frameworks, the Master Cell Seed is a fundamental requirement for ensuring the safety and consistency of vaccine production. It provides a fully characterized, standardized, and pathogen-free cell line that must be used for all future manufacturing batches. This rigorous standardization ensures that every dose of the vaccine is produced under identical conditions, minimizing variability and preventing the accidental introduction of contaminants from other cell sources during the industrial scale-up.

**245.** (A) Both A and R are true and R is the correct explanation of A.
Finding the precise minimum immunizing dose during pre-clinical trials is a critical engineering and biological challenge. This is because excessive antigen payloads can lead to negative consequences, such as the induction of immune tolerance or a higher risk of adverse site reactions. Furthermore, using more antigen than necessary increases overall production costs. Therefore, identifying the lowest effective dose ensures that the final vaccine is safe, affordable, and provides a robust and protective response.

**246.** (A) Lyophilized live vaccines
In post-production quality control, a test for residual moisture is mandatory specifically for lyophilized live vaccines. This is because the stability and shelf life of the dry product are highly dependent on the amount of water remaining. If too much moisture is present, it

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**216.** (C) I and III
Particulate delivery systems like liposomes and polymeric particles offer several advantages for modern vaccines. These structures can protect delicate antigens from proteolytic degradation and often enhance their uptake by antigen-presenting cells because they mimic the size and dimensions of real pathogens. While some systems are complex, they do not all require Freund's Complete Adjuvant to function effectively, making them versatile tools for designing many different types of vaccines.

**217.** (A) In the follicle-associated epithelium of Peyer's patches and NALT
Targeted mucosal delivery methods aim to exploit the specialized sampling capacity of M-cells, which are found in the follicle-associated epithelium. These cells are located in areas like the Peyer's patches of the intestine and the nasopharynx-associated lymphoid tissue. By using ligands that bind to M-cells, researchers can ensure that vaccine antigens are efficiently transported across the epithelial barrier, leading to the induction of a strong local and systemic response.

**218.** (B) Excessive shear forces generated by aggressive agitation can physically rupture and kill mammalian cells
In large-scale bioreactor operations, monitoring and controlling shear stress is vital for the health of the cell culture. This is because mammalian cells lack a rigid cell wall and are sensitive to physical forces. Excessive shear forces generated by aggressive agitation or aeration can physically rupture and kill these cells. Therefore, engineering the reactor to provide adequate mixing while minimizing shear is essential for maintaining high productivity in vaccine manufacturing.

**219.** (A) Protein aggregation and surface adsorption
Surfactants like Polysorbate 80 are common excipients included in vaccine formulations for their protective properties. Their primary role is to prevent physical degradation mechanisms such as protein aggregation and adsorption to the surface of the container. By reducing the surface tension, these molecules help maintain the antigen in a stable and soluble state, ensuring that the vaccine remains potent and safe during storage and transport to the field.

**220.** (A) Real-time and accelerated stability testing
The expiry date for any veterinary vaccine is established based on rigorous stability testing. Manufacturers perform both real-time and accelerated studies to determine how long the product remains potent and safe under recommended storage conditions. This data ensures that the vaccine will still provide protection when administered, provided it has been handled correctly throughout the cold chain. This testing is a fundamental requirement for regulatory approval and market release.

**221.** (A) The capacity of the vaccine to stimulate a measurable humoral and/or cellular immune response
In the context of clinical trials, immunogenicity is a specific term used to describe the primary performance of a vaccine. It refers to the product's capacity to stimulate a measurable humoral or cellular immune response in the target species. Measuring parameters like antibody titers or T-cell activation allows researchers to determine if the vaccine is successfully engaging the immune system, which is a necessary prerequisite for establishing protective efficacy.

**222.** (A) Both A and R are true and R is the correct explanation of A.
In vitro potency tests are increasingly favored over animal-based challenge models for the quality control of individual vaccine batches. These assays, such as quantifying antigen mass via ELISA, are generally faster, more reproducible, and align with modern ethical standards for animal welfare. By providing a precise measurement of the active components, these tests ensure that each batch is consistent and effective while reducing the overall use of laboratory animals.

**223.** (C) It establishes long-lasting immunological memory
Passive immunization is a useful tool for providing immediate protection by administering pre-formed antibodies, such as equine tetanus antitoxin. However, it is incorrect to suggest that this method establishes long-lasting immunological memory. Because the host's own B and T cells are not activated, the protection only lasts as long as the transferred antibodies remain in the circulation. Once they degrade, the animal is again vulnerable to infection by the pathogen.

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targeting the protein to a specific cellular organelle is often necessary to maximize yield. Researchers frequently target these proteins to the endoplasmic reticulum, as this often results in the highest levels of stable accumulation. This environment provides the necessary conditions for proper folding and post-translational modifications, ensuring that the resulting antigen is both abundant and effective for use in the final vaccine formulation.

**255.** (A) Endocytosis of the LNP, endosomal escape of mRNA into the cytosol, translation of mRNA by host ribosomes into protein, presentation of antigen on MHC molecules
The successful action of an mRNA vaccine follows a precise molecular cascade after it is administered into the host. It begins with the endocytosis of the lipid nanoparticle by host cells, followed by the escape of the mRNA into the cytosol. Once in the cytosol, the host's ribosomes translate the mRNA into the target protein antigen. These proteins are then processed and presented on MHC molecules to initiate a robust and protective immune response.

**256.** (C) They exhibit exceptional immunogenicity as naked, standalone molecules
Synthetic peptide vaccines are a highly stable and safe platform that requires precise knowledge of a pathogen's epitopes. However, it is incorrect to suggest that they exhibit exceptional immunogenicity as naked, standalone molecules. Because they are small and often lack the necessary signals for full immune activation, they typically require the use of carrier proteins and potent adjuvants to overcome this limitation and provide an effective level of protection for the host animal.

**257.** (A) Widespread vaccination while preserving the "disease-free" international trade status of a region
The implementation of a DIVA strategy provides a major advantage for disease eradication efforts. It enables widespread vaccination of animal populations while still preserving the "disease-free" status required for international trade. Because diagnostic tests can distinguish between vaccinated and infected animals, countries can control outbreaks without the need for large-scale culling, protecting both animal health and the economic interests of farmers and the entire livestock industry during a critical disease outbreak.

**258.** (A) To prevent the induction of autoimmune responses against host tissues
A critical step in the reverse vaccinology screening process is filtering out potential antigens that share significant homology with the host genome. This is essential for safety, as identifying and using such proteins in a vaccine could lead to the induction of autoimmune responses against the animal's own tissues. By ensuring that the vaccine targets are unique to the pathogen, researchers can minimize the risk of selF-reactivity and develop a safer and more effective product.

**259.** (A) Determine the high-resolution, three-dimensional structure of complex viral glycoproteins in their native state without crystallization
Cryo-electron microscopy has revolutionized the field of structural vaccinology by allowing researchers to see antigens in a new way. This technology enables the determination of the high-resolution, three-dimensional structure of complex viral glycoproteins in their native state without the need for difficult protein crystallization. This information is vital for the rational design of vaccines that can effectively target the most important parts of a pathogen and provide a high level of protection for animals.

**260.** (A) They can accurately predict the magnitude of the antibody response months later
The use of transcriptomics in systems vaccinology provides a powerful tool for evaluating new products. Identifying early molecular signatures after vaccination is highly valuable because these patterns can accurately predict the magnitude and quality of the antibody response months later. This allows researchers to identify effective vaccine candidates much faster than traditional methods, which is particularly important during emergencies where a rapid response is needed to control the spread of a new infectious disease.

**261.** (D) A is false but R is true.
Adversomics does not seek to abandon mass vaccination; instead, it aims to make the process safer through the use of immunogenetic profiling. By identifying specific genetic markers that are associated with a higher risk of adverse events, researchers can pinpoint susceptible

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immune system might also mount a response against the host's own immunoglobulins. This could potentially lead to the development of auto-immune diseases, which is a significant safety concern that must be carefully evaluated during the development of these platforms.

**232.** (A) Small peptides act as haptens and fail to reliably recruit T-cell help without the carrier's T-cell epitopes
Synthetic peptide vaccines typically require coupling to a large carrier protein to be effective. This is because small peptides often act as haptens, meaning they can be recognized by B cells but fail to reliably recruit the necessary T-cell help on their own. The carrier protein provides the essential T-cell epitopes that stimulate a full adaptive response, ensuring the production of high-affinity antibodies and the establishment of long-term immunological memory.

**233.** (A) The purified marker antigen that is present in the wild-type virus but deleted from the vaccine
In a DIVA strategy, the indirect ELISA used for diagnostic testing must be able to distinguish between animals that have been vaccinated and those that were naturally infected. To achieve this, the assay plate is coated with a purified version of the marker antigen that is present in the wild-type virus but was deleted from the vaccine strain. Antibodies against this specific protein will only be found in naturally infected animals.

**234.** (A) Genes shared by all sequenced strains of a particular bacterial species
Reverse vaccinology uses the concept of the pan-genome to identify the best potential vaccine targets across a bacterial species. In this framework, the core genome specifically refers to the set of genes that are shared by every sequenced strain of the pathogen. Identifying antigens from this core group is vital for creating a vaccine that can provide universal protection against all different versions and variants of the disease found in the field.

**235.** (A) They expose highly conserved, neutralization-sensitive epitopes that are otherwise hidden after the protein undergoes conformational rearrangement
Computationally designed pre-fusion stabilized antigens are highly effective because of their structural properties. By locking the protein into this specific shape, the vaccine ensures that it exposes highly conserved epitopes that are otherwise hidden or lost after the protein undergoes a conformational change during infection. These exposed epitopes are the primary targets for neutralizing antibodies, leading to a much more potent and broader immune response that can effectively block the pathogen.

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**236.** (C) Systems vaccinology
Systems vaccinology is an interdisciplinary field that integrates data from several sources, including genomics, transcriptomics, and proteomics, to create a comprehensive model of the host response to a vaccine. This approach uses advanced mathematical tools to identify the complex patterns and interactions that occur within the immune system. The ultimate goal is to understand how these molecular changes lead to protection and to use this knowledge to design more effective vaccines.

**237.** (A) The Major Histocompatibility Complex (MHC)
Adversomics research has overwhelmingly shown that the host's genetic background is a major factor in determining how an individual responds to a vaccine. Specifically, the Major Histocompatibility Complex is the genetic region most frequently implicated in idiosyncratic or severe adverse reactions. Because these molecules play a central role in presenting antigens to the immune system, variations in their genes can lead to significant differences in safety and efficacy across a population.

**238.** (A) An antigen specifically directs the adaptive immune response, while an adjuvant non-specifically enhances the magnitude and type of that response
There is a fundamental functional difference between the two main components of a modern vaccine. The antigen is the specific part of the pathogen that directs the adaptive immune response, ensuring that the antibodies and T cells produced are targeted correctly. In contrast, the adjuvant is an additive that non-specifically enhances the overall magnitude and type of that response. Together, they ensure that the vaccine is both specific and powerful enough to provide protection.

**208.** (D) I, II, and III
Synthetic peptide vaccines often face challenges related to their physical structure, as small linear sequences frequently lack a rigid three-dimensional shape in solution. This loss of native conformation can limit the production of high-affinity neutralizing antibodies against important epitopes. To address this, researchers use strategies like cyclization to constrain the peptide into a more stable, native-like shape, which significantly improves the resulting immune response.

**209.** (A) False positives, which would lead to the unnecessary culling of healthy vaccinated animals
For a DIVA strategy to be effective in a disease control program, the companion diagnostic assay must be highly specific. If the assay produces false positives, it could lead to the unnecessary culling of healthy vaccinated animals, which would be a major programmatic and economic failure. Therefore, rigorous validation is required to ensure the test only identifies natural infection and does not react with the vaccine itself.

**210.** (A) NetMHC / IEDB tools
Reverse vaccinology relies on sophisticated computational tools to screen entire pathogen genomes for the most effective antigens. Tools like NetMHC and other IEDB resources are integral to this process, as they are used to predict the binding affinity of various peptides to MHC Class I and II molecules. Identifying these epitopes allows researchers to focus their efforts on proteins that are most likely to stimulate T-cell responses.

**211.** (A) Structural vaccinology
The emergence of AlphaFold, an advanced AI system for protein structure prediction, has profoundly accelerated the field of structural vaccinology. By providing highly accurate three-dimensional models of antigens from simple amino acid sequences, AlphaFold allows researchers to bypass time-consuming laboratory experiments like crystallography. This information is invaluable for rationally designing stable vaccines that expose the best epitopes for neutralizing antibodies, even for complex or poorly understood pathogens.

**212.** (A) Predict the final antibody titer without waiting months for the serological response
In the field of systems vaccinology, researchers use transcriptomics to identify specific molecular signatures shortly after a vaccine is given. The great value of these signatures is that they can accurately predict the magnitude and quality of the final antibody response months before it would normally be measured. This early prediction allows for much faster evaluation of vaccine candidates and a deeper understanding of the mechanisms of immunity.

**213.** (A) First exposure to antigen, IgE production, IgE binds to mast cells/basophils, second exposure cross-links IgE, degranulation (histamine release)
The molecular cascade leading to anaphylaxis follows a precise chronological sequence. It begins with the first exposure to an antigen, which triggers the production of IgE antibodies. These antibodies then bind to the surface of mast cells and basophils. Upon a second exposure, the antigen cross-links the bound IgE, leading to rapid degranulation and the release of histamine. This process causes the immediate and severe symptoms of the reaction.

**214.** (A) Freund's Complete Adjuvant (FCA)
Freund's Complete Adjuvant is a well-known tool for inducing powerful immune responses in laboratory settings. It consists of a water-in-oil emulsion that contains killed mycobacteria. These bacterial components provide a strong stimulus that activates the innate immune system and drives a potent cell-mediated defense. While highly effective, its use is often restricted in food-producing animals because it can cause severe local reactions and tissue damage at the injection site.

**215.** (A) Natural Killer (NK) cells and Th1 cells
Interferon-gamma is a critical cytokine that acts as a powerful immunomodulator during the early stages of an immune response. Its principal sources are Natural Killer cells and the Th1 subset of T lymphocytes. By secreting this molecule, these cells help activate macrophages and promote a cellular defense that is essential for clearing intracellular pathogens. This cytokine is often a key target for measurement when evaluating the efficacy of viral vaccines.

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can lead to chemical degradation and a loss of potency over time. Ensuring that the moisture levels are within strict limits is essential for maintaining the vaccine's effectiveness until use.

**247.** (A) Toxoids stimulate active immunity and the formation of memory B cells for long-term protection
The primary rationale for using a tetanus toxoid vaccine for long-term prophylaxis is its ability to stimulate active immunity. This process leads to the formation of specialized memory B cells that can provide protection for many years. Unlike antitoxins, which only provide immediate and temporary defense, the toxoid ensures that the animal's own immune system is primed to respond rapidly if the tetanus toxin is encountered again in the future through an injury.

**248.** (A) They possess an inherent risk of residual virulence or reversion, particularly in non-target or immunocompromised hosts
The historical experience with certain vaccines highlights a fundamental disadvantage of classical live-attenuated platforms. Because these products contain a living, though weakened, organism, they possess an inherent risk of residual virulence or reversion to a more harmful state. This risk is particularly significant for non-target species or immunocompromised hosts, as seen with some Brucella strains. This emphasizes the need for careful safety monitoring and the development of more stable, rationally designed vaccine alternatives.

**249.** (A) I and II
The mucosal immune response is characterized by the production of secretory IgA, which is a complex molecule consisting of a dimer linked by a J chain and a secretory component. This specific component is vital because it protects the antibody from proteolytic cleavage in the harsh mucosal environment. While intranasal vaccines are highly effective at inducing this defense, it is incorrect to assume that systemic intramuscular vaccines are universally superior for local mucosal protection.

**250.** (A) P-3, Q-4, R-2, S-1
Different vaccine types have defining characteristics that determine how they engage the immune system. Subunit vaccines use isolated components, while toxoids consist of detoxified exotoxins. Live-attenuated vaccines contain weakened pathogens that can replicate, and inactivated whole-agent vaccines use pathogens that have been completely killed by physical or chemical means. Understanding these categories is essential for choosing the right platform to induce the specific type of immunity needed to protect against a particular disease.

**251.** (A) The physical packaging constraints of the icosahedral viral capsid
Recombinant Adenovirus vectors are popular for vaccine delivery, but their use is subject to a strict engineering limitation. The size of the foreign gene insert is limited by the physical packaging constraints of the viral capsid. If the inserted DNA is too large, the genome will not fit inside the icosahedral shell, preventing the formation of mature, infectious particles. This constraint necessitates the careful selection of target antigens and the optimization of the vector's genome.

NODIA

**252.** (A) To create a negative marker DIVA vaccine strain that lacks the immunodominant O-antigen
Engineering bacterial pathogens to create "rough" mutants by deleting genes for the O-antigen side chain is a strategic approach for rational attenuation. The primary purpose of this modification is to create a negative marker for a DIVA strategy. Since the vaccine strain lacks this immunodominant surface antigen, diagnostic tests can easily identify naturally infected animals by looking for antibodies against the missing O-antigen, allowing for more effective disease control and eradication programs in herds.

**253.** (A) It acts as the binding site for the eukaryotic ribosome to ensure efficient translation initiation
In the design of DNA vaccines, the Kozak sequence is a critical regulatory element included just upstream of the antigen coding region. This specific sequence acts as the binding site for the eukaryotic ribosome and is essential for ensuring efficient translation initiation in the host's cells. Without this sequence, the expression of the protein antigen would be significantly reduced, leading to a much weaker and potentially ineffective immune response against the target pathogen after administration.

**254.** (A) Endoplasmic reticulum
When expressing recombinant viral glycoproteins in transgenic plants for vaccine production,

**224.** (A) Maternal antibodies neutralize the live vaccine virus before it can replicate and induce an immune response
Interference is a common problem when using traditional live-attenuated vaccines in young animals. This occurs when maternal antibodies, received through colostrum, neutralize the vaccine virus before it has a chance to replicate and stimulate the calf's own immune system. This interference can prevent the development of a long-lasting memory response, which is why timing the first vaccination correctly is critical for success in veterinary herd health programs.

**225.** (A) P-2, Q-3, R-4, S-1
The immune response follows specific kinetic patterns depending on previous exposure. The primary IgM response peaks early and then declines. The primary IgG response develops more slowly but eventually dominates. In contrast, the secondary IgG response is immediate and reaches much higher titers. Finally, memory T cell responses are characterized by rapid and massive clonal expansion upon re-exposure, providing the foundation for long-term protection against the specific pathogen encountered.

**226.** (B) By transfecting host cells which synthesize the antigen in the cytosol, leading to proteasomal processing and MHC Class I presentation
DNA vaccines are uniquely effective at inducing cytotoxic T-cell responses through a specific biological pathway. After administration, the plasmid DNA transfects the host's own cells, which then synthesize the target antigen directly in their cytosol. These proteins are then processed by proteasomes and presented on MHC Class I molecules. This endogenous presentation mimics a real viral infection, which is the signal required to activate naive CD8+ T cells for protection.

**227.** (B) II and III
Recombinant Listeria monocytogenes is a powerful vaccine vector due to its unique life cycle. Unlike many bacteria, Listeria can naturally escape from the host cell's phagosome into the cytosol. This escape allows the delivered antigens to enter the endogenous processing pathway and be presented on MHC Class I molecules, driving a potent CD8+ T cell response. Attenuation is typically achieved by deleting essential virulence genes like actA to ensure safety.

**228.** (A) The genetic basis of attenuation is defined and the risk of back-mutation to wild-type is virtually eliminated
Rationally attenuated viral vaccines offer several advantages over those created through classical serial passage. Because they are developed by deleting specific genes with a known function, the genetic basis of their weakness is clearly defined. This precision virtually eliminates the risk of the pathogen reverting to a wild-type state through back-mutation. This high level of genetic stability ensures that the vaccine remains safe and predictable for use in diverse animal populations.

**229.** (A) SV40 or human Cytomegalovirus (hCMV) immediate-early promoter
Plasmid DNA vaccines are complex constructs that include several regulatory elements. In addition to immunostimulatory CpG motifs, they require a powerful promoter to ensure high levels of antigen expression in mammalian cells. The human cytomegalovirus immediate-early promoter is ubiquitous in these constructs because of its robust activity. This promoter drives the host cell's machinery to produce the protein antigen needed to initiate both humoral and cell-mediated immune responses for protection.

**230.** (A) Identify the protective epitope, chemically synthesize the peptide, conjugate the peptide to a carrier protein, mix with an appropriate adjuvant
Developing a synthetic peptide vaccine involves a precise series of steps. First, the most protective epitope on the pathogen must be identified. This sequence is then chemically synthesized and conjugated to a large carrier protein to improve its immunogenicity. Finally, the resulting conjugate is mixed with an appropriate adjuvant. This entire process ensures that the final product can effectively stimulate the immune system and provide lasting protection against the target disease.

**231.** (A) May induce a strong immune response against the host's own immunoglobulins, potentially leading to autoimmunity
A major theoretical challenge with anti-idiotypic vaccines is the potential for inducing autoimmunity. Because these vaccines consist of antibodies that mimic the structure of a pathogen's antigen, there is a risk that the

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subpopulations. This knowledge allows for the design of safer formulations or personalized immunization protocols, ensuring that the benefits of vaccination can be achieved with minimal risk to the health of individual animals and herds.

**262.** (A) Inducing lysosomal destabilization upon phagocytosis, which triggers the NLRP3 inflammasome to secrete IL-1beta
Aluminum-based adjuvants are the most common additives in modern vaccines, and they operate through a specific cellular mechanism to boost immunity. After being taken up by immune cells, they cause lysosomal destabilization, which triggers the NLRP3 inflammasome. This leading to the secretion of the pro-inflammatory cytokine Interleukin-1beta. This signaling pathway is critical for activating the innate immune system and creating the environment necessary for a strong and effective adaptive response to the vaccine antigen.

**263.** (A) Cytokine genes ensure sustained, localized expression, overcoming the extremely short halF-life of injected proteins
When using cytokines as vaccine enhancers, delivering the cytokine gene via a plasmid or viral vector is often superior to injecting the protein directly. This approach ensures sustained and localized expression of the cytokine at the site of immunization, which is essential because many of these molecules have an extremely short in vivo halF-life. By maintaining a steady level of signaling, these genetic constructs can more effectively steer the immune response in the desired direction.

**264.** (A) I, II, and III
Immune Stimulating Complexes are advanced cage-like particles that represent a versatile platform for vaccine delivery. They are typically 40 nm in diameter and are composed of saponins, cholesterol, and phospholipids combined with the target antigen. These structures are highly efficient at delivering antigens directly into the host cell's cytosol, which is the required step for presentation on MHC Class I molecules and the activation of a strong cytotoxic T-cell response against various pathogens.

**265.** (A) In a batch culture, no nutrients are added after inoculation; in a fed-batch culture, specific nutrients are added to prolong the growth phase
In industrial vaccine manufacturing, there is a fundamental operational difference between batch and fed-batch bioreactor cultures. In a simple batch culture, all the nutrients are added at the start, and no more are provided during the run. In contrast, a fed-batch culture involves the periodic addition of specific nutrients to prolong the growth phase of the cells. This approach leads to significantly higher cell densities and a much larger final yield of the vaccine antigen.

**266.** (A) Net surface charge at a specific pH
Ion-exchange chromatography is a cornerstone technique used in the downstream purification of recombinant vaccine antigens. This method fundamentally exploits the net surface charge of the protein at a specific pH to separate it from other contaminants in the mixture. By carefully controlling the conditions, researchers can ensure that only the target antigen binds to the chromatography matrix, while other cellular debris and unwanted proteins are washed away, resulting in a highly pure final product.

**267.** (C) Preservatives are heavily utilized in lyophilized single-dose live vaccines to keep the virus alive
Preservatives are essential components of multi-dose vaccine vials to prevent microbial contamination after the stopper is punctured. Compounds like thimerosal have a long history of safe use for this purpose. However, it is incorrect to suggest that they are heavily utilized in lyophilized single-dose live vaccines to keep the virus alive. In fact, high concentrations of preservatives can negatively impact the potency of many live vaccines, so they are generally avoided in those formulations.

**268.** (A) P-4, Q-3, R-1, S-2
Various regulatory terms define the standards for vaccine production and safety. Good Manufacturing Practice ensures consistent quality control, while pharmacopeias provide official standardized procedures. Target animal safety evaluates adverse effects in the intended host species, and batch release is the final process of verifying that each lot meets all criteria before sale. Adhering to these rigorous standards is essential for protecting animal health and

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only the most effective antigens proceed further into the vaccine development process, saving time and resources while improving the chances of final success.

**284.** (A) Delete or mask this loop to redirect the response toward conserved, sub-dominant neutralizing epitopes
Structural vaccinology aims to optimize the design of antigens to induce the most effective immune response. If an antigen possesses a highly flexible, non-neutralizing loop, a structural approach might involve deleting or masking this specific part. This strategy helps redirect the immune system's focus toward more important, conserved, and sub-dominant neutralizing epitopes that are otherwise hidden. This engineering leads to a more targeted and potent vaccine that can better protect against complex or highly mutable pathogens.

**285.** (A) The precise subsets and activation states of the diverse cellular immune compartments responding to the vaccine
In the field of vaccinomics, high-dimensional flow cytometry is an essential tool for evaluating the complexity of the host's defense. This technology allows researchers to analyze the precise subsets and activation states of the diverse cellular immune compartments that respond to a vaccine. By providing such a detailed picture of the cellular response, researchers can identify the specific immune signatures that correlate with protection, leading to a deeper understanding of how vaccines work and how to improve them.

**286.** (A) To screen out and exclude genetically susceptible individuals from receiving a specific vaccine formulation
The fundamental goal of identifying an adversomic biomarker before starting a vaccination campaign is to improve overall safety. By finding specific genetic markers that predict a higher risk of severe adverse reactions, researchers can screen out and exclude genetically susceptible individuals from receiving a particular formulation. This personalized approach ensures that vaccines are administered more safely and allows for the development of alternative strategies for those who may be at risk, ultimately protecting the entire population.

**287.** (A) I, II, and IV
Toll-Like Receptor agonists are effective modern adjuvants because they bridge the gap between the innate and adaptive immune systems. For example, monophosphoryl lipid A is a detoxified derivative of LPS that acts as a potent TLR4 agonist. These molecules typically skew the immune response toward a Th1 profile, which is vital for cell-mediated immunity. Using these agonists in vaccines helps to ensure a strong and targeted defense against many intracellular pathogens and various viral infections.

**288.** (A) P-2, Q-4, R-3, S-1
Cytokines serve as critical signals that direct the development of the immune response. Interleukin-12 drives Th1 differentiation and cellular immunity, while Interleukin-4 promotes Th2 activity and humoral responses. GM-CSF enhances the recruitment and maturation of dendritic cells, and Interleukin-15 is essential for the maintenance and survival of long-lived memory CD8+ T cells. Matching these molecules to their targeted effects is a key part of rational vaccine design to ensure the most effective protection.

NODIA

**289.** (A) The rate of polymer degradation and the subsequent kinetics of antigen release
When designing a PLGA polymeric microsphere vaccine, the choice of materials is critical for controlling performance. Adjusting the ratio of lactide to glycolide polymers strictly determines the rate at which the polymer degrades in the host's body. This, in turn, controls the subsequent kinetics of the antigen release. By tailoring this ratio, researchers can engineer a vaccine that provides sustained exposure to the antigen, potentially reducing the number of booster doses needed for immunity.

**290.** (A) They possess mucoadhesive properties and can respond to physiological triggers like pH or temperature
The integration of nanotechnology into vaccinology has led to the development of advanced delivery vehicles like nanogels. These structures are ideal for mucosal delivery because they possess unique physicochemical properties, such as mucoadhesion. Furthermore, nanogels can be engineered to respond to physiological triggers like changes in pH or temperature. This sensitivity allows them to release the vaccine antigen precisely at the mucosal surface, leading

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exceedingly strong polyhedrin promoter to drive the expression of recombinant proteins. This system frequently yields very high levels of protein and possesses the machinery for complex mammalian post-translational modifications. This ensures that the resulting antigens are correctly folded and highly immunogenic, making them effective tools for developing many different types of modern vaccines.

**277.** (A) An infectious complementary DNA (cDNA) clone of the viral genome
Generating a rationally attenuated virus through reverse genetics is a sophisticated process that begins at the molecular level. It inherently requires starting from an infectious complementary DNA clone of the viral genome. This cloned DNA allows researchers to introduce precise mutations or deletions before rescuing the live virus in cell culture. This technology is a powerful tool for designing vaccines with a clearly defined safety profile, as the genetic changes are controlled and stable throughout production.

**278.** (A) Bacterial and viral DNA
Plasmid DNA vaccines often include unmethylated CpG motifs because of their powerful immunostimulatory properties. These specific sequences structurally mimic the DNA that is naturally found in bacteria and viruses, which is often unmethylated compared to mammalian DNA. The immune system recognizes these motifs as a "danger signal" through Toll-Like Receptor 9, triggering an innate response that helps activate and steer the adaptive immune response toward a more effective and protective profile for the host animal.

**279.** (A) Regulatory challenges regarding dosage standardization and environmental contamination concerns
Despite their potential benefits, several significant obstacles prevent the widespread commercialization of edible plant-based vaccines. One of the most prominent challenges involves regulatory concerns regarding the standardization of dosage and the potential for environmental contamination. Ensuring that every animal receives a consistent amount of antigen through a fruit or leaf is difficult. Furthermore, preventing the accidental spread of vaccine genes to wild plants or other crops is a major concern for regulatory authorities and manufacturers.

**280.** (C) Ab2 acts purely as an immunosuppressive agent and never stimulates immunity
The Jerne immune network theory provides a complex model of how antibodies interact to regulate the immune system. While it postulates a network of idiotypes and anti-idiotypic antibodies, it is incorrect to suggest that Ab2 antibodies act purely as immunosuppressive agents. In fact, specific Ab2 beta antibodies are the key components of anti-idiotypic vaccines because they resemble the nominal antigen and can actively stimulate a protective immune response against the original pathogen, providing a safe alternative for immunization.

**281.** (A) Hydrophilic, highly mobile, and located on the surface of the native protein
The process of mapping B-cell epitopes for synthetic peptide vaccines relies on identifying sequences that are most accessible to the immune system. Researchers look for regions that are hydrophilic, highly mobile, and located on the surface of the native protein. These properties ensure that the epitopes can be easily recognized by B-cell receptors and circulating antibodies. By targeting these exposed areas, scientists can design vaccines that are more effective at inducing a potent and protective immune response.

**282.** (A) Non-essential for in vitro replication and highly immunogenic
For a DIVA strategy for infectious bovine rhinotracheitis to be successful, the chosen marker protein must have specific biological properties. The gE glycoprotein in the wild-type virus must be non-essential for the virus to replicate in cell culture, allowing for the manufacture of the vaccine. Crucially, it must also be highly immunogenic so that all naturally infected animals will develop a clear antibody response that can be easily detected by the companion diagnostic test.

**283.** (A) Cloning, expressing the recombinant proteins, and screening them for immunogenicity and protective efficacy
In a reverse vaccinology project, identifying potential antigens in silico is only the first step. The mandatory next step to validate these candidates involves cloning and expressing the recombinant proteins in the laboratory. These proteins must then be screened for their immunogenicity and protective efficacy in animal models. This rigorous validation ensures that

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maintaining the public's trust in veterinary pharmaceutical products.

**269.** (A) Laboratory immunogenicity and safety, dose-determination, large-scale field efficacy, post-marketing surveillance
The development of a new veterinary vaccine follows a specific lifecycle of clinical trials to ensure safety and efficacy. It begins with laboratory immunogenicity and safety studies in a small group, followed by dose-titration to find the optimal payload. Next, large-scale field efficacy trials are conducted under natural conditions. Finally, after the vaccine is licensed, post-marketing surveillance is performed to monitor its long-term performance and identify any rare adverse events in the population.

**270.** (A) That the final product is free from contaminating adventitious microorganisms, chemicals, or cellular debris
In the context of post-production quality control, a purity test is a mandatory procedure performed on every batch of vaccine. This specific test verifies that the final product is free from any contaminating adventitious microorganisms, unwanted chemicals, or cellular debris from the manufacturing process. Ensuring high levels of purity is essential for safety, as it prevents the accidental introduction of harmful substances or pathogens when the vaccine is administered to animals in the field.

**271.** (A) Purified recombinant subunit proteins
Every vaccine platform represents a balance between safety and efficacy for the target population. Purified recombinant subunit proteins are generally considered to be the safest class of vaccines because they contain zero infectious material and cannot replicate. However, because they consist of only a small part of the pathogen, they often have the lowest intrinsic immunogenicity. To provide a high level of protection, these vaccines typically require the use of potent adjuvants and multiple booster doses.

**272.** (A) Both A and R are true and R is the correct explanation of A.
Oral vaccines are uniquely suited for inducing a strong local defense at mucosal surfaces. This is because the gut-associated lymphoid tissue contains specialized M-cells and dendritic cells that sample antigens from the intestinal lumen. These cells then promote IgA class switching in B cells, leading to the production of secretory IgA. This specific antibody isotype is essential for neutralizing pathogens before they can cross the epithelial barrier, providing a critical first line of defense.

**273.** (A) Induce a secondary immune response characterized by rapid production of high-affinity IgG and expansion of memory pools
The primary immunological rationale for administering booster vaccinations is to induce a secondary immune response. This response is much more efficient than the initial encounter, characterized by the rapid production of high-affinity IgG antibodies and a massive expansion of the memory B and T cell pools. By reinforcing the immune system's memory, boosters ensure that the animal maintains a high level of protection over a longer period, reducing the risk of infection and disease.

**274.** (A) Effectively inactivates nucleic acids with minimal alteration of structural surface epitopes
In the manufacture of inactivated whole-agent vaccines, beta-propiolactone is often preferred over formaldehyde for certain viral targets. This is because BPL effectively inactivates the pathogen's nucleic acids while causing minimal alteration to the important structural surface epitopes. Preserving these proteins in their native conformation is critical for ensuring that the resulting vaccine remains highly immunogenic and can stimulate the production of potent neutralizing antibodies in the host animal after administration.

NODIA

**275.** (A) Dendritic cells
During the primary immune response to a vaccine or pathogen, the activation of naive T-cells is a critical step that requires a specialized cell type. Dendritic cells are the only antigen-presenting cells that are uniquely capable of performing this task. They capture the antigen, process it, and then travel to the lymph nodes where they provide the necessary signals to naive T cells. This efficient priming is essential for launching a successful and protective adaptive response.

**276.** (A) I, II, and IV
Eukaryotic expression systems utilizing baculovirus are highly valued for vaccine production because they offer several advantages. These viruses infect insect cells and use the

to a more effective and localized immune response against many common pathogens.

**291.** (A) Tangential Flow Filtration
In the downstream processing of large-scale recombinant vaccines, clarification is a vital step to ensure the quality of the final product. Tangential Flow Filtration is a technique frequently used for this purpose. This method effectively removes large cellular debris and unwanted components while allowing the soluble protein product to pass into the permeate. This efficient separation is essential for preparing the antigen for further purification and concentration steps in the manufacturing process for animals.

**292.** (B) The system of transporting and storing vaccines within a recommended temperature range from manufacture to administration
The cold chain is a critical logistical system in the distribution of veterinary vaccines. It consists of the entire sequence of transporting and storing the products within a strictly recommended temperature range, from the moment of manufacture until they are administered in the field. Maintaining the cold chain is essential for preserving the potency and safety of the vaccine, as exposure to extreme heat or cold can rapidly degrade the biological components and make them ineffective.

**293.** (A) The quantitative measure of the capacity of the product to achieve its intended immunologic effect
According to pharmacopeial guidelines, the potency test is a mandatory quality control procedure performed on every batch of a veterinary vaccine. This test provides a quantitative measure of the capacity of the product to achieve its intended immunologic effect in the target species. Ensuring that each batch meets established potency standards is essential for the vaccine to be effective in providing protection against the specific disease it was designed to target in animal populations.

**294.** (A) Target animal safety studies, dose titration, laboratory challenge efficacy, field efficacy trials
Completing the development of a veterinary vaccine follows a logical sequence of studies to ensure performance. It typically begins with target animal safety studies to identify any adverse effects. This is followed by dose titration to find the optimal payload and laboratory challenge studies to evaluate efficacy. Finally, large-scale field trials are conducted to measure performance under natural conditions. This comprehensive approach is essential for meeting regulatory requirements and ensuring the product is safe and effective.

**295.** (A) Both A and R are true and R is the correct explanation of A.
The limulus amebocyte lysate test is a fundamental quality control assay for any recombinant vaccine produced in Escherichia coli systems. This is because E. coli is a Gram-negative bacterium that naturally contains endotoxins in its cell walls. The LAL test is highly sensitive for detecting these residual lipopolysaccharides in the final purified product. Ensuring their absence is critical for safety, as endotoxins can cause severe inflammatory reactions or fever in vaccinated animals during immunization.

**296.** (A) CD80/86 (B7-1/B7-2)
In mammalian cellular immune responses, the activation of naive T-cells is a highly controlled process that requires two distinct signals. The first is the recognition of the antigen-MHC complex. The second is a required costimulatory signal on the surface of the antigen-presenting cell. Specifically, the binding of CD80 or CD86 molecules to the CD28 receptor on the naive T cell is essential for providing the full signal needed for activation, proliferation, and differentiation.

**297.** (A) I, II, and IV
Traditional killed vaccines are created using chemical or physical inactivation methods and represent zero risk of shedding live pathogens into the environment. These vaccines are highly safe and often require a prime-boost regimen to maintain protective immunity. While effective at inducing antibodies, they generally do not stimulate powerful cytotoxic CD8+ T-cell responses natively. Therefore, they are often used with adjuvants to improve their overall performance and provide a broader defense for the host animal.

**298.** (A) They have lost the ability to replicate efficiently in most mammalian cells, undergoing an abortive infection
Poxvirus vector platforms offer an inherently safe profile for use in many different host species. This safety is largely because many of

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these specialized vectors have lost the ability to replicate efficiently in most mammalian cells. Instead, they undergo what is known as an abortive infection. This means the virus can enter the cell and express the delivered antigen without completing its full life cycle or spreading, which prevents accidental infection in vaccinated animals.

**299.** (A) It attenuates the virus by reducing its ability to replicate in non-dividing cells like neurons

In the development of marker vaccines using targeted gene deletion, the thymidine kinase gene is a common target for removal. Deleting this specific gene attenuates the virus by significantly reducing its ability to replicate in non-dividing cells, such as neurons. This modification makes the vaccine strain safer for use in host animals, particularly for viruses that can establish latent infections. This rational design ensures that the vaccine provides protection without the risk of causing disease.

NODIA

**300.** (A) A surrogate metric associated with protection from disease

The ultimate objective of systems vaccinology and multi-omics profiling is to identify precise correlates of protection for each vaccine. This fundamentally means finding a specific biological biomarker, such as a particular antibody titer or cellular signature, that is statistically associated with protection from disease. Having a clear correlate allows researchers and manufacturers to measure the efficacy of a vaccine more accurately and quickly, ensuring that animal populations are successfully protected against the target pathogen in the field.

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